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Populism . . . elitism . . . or a middle way?

THE newly formed Science, Technology and Society Association started life with a suitably large splash last week in London by having Mr Tony Benn, UK Secretary of State for Energy and the association's president, talk on the democratic control of science and technology. The association has emerged from the SISCON (Science in a Social Context) project which, over the past few years, has brought together various groups in higher education in Britain interested in the broader issues of science. The plan now is to reach beyond the academic world and not just into other areas of education. Its first newsletter speaks of hoping to draw members from industrial managers, trade unionists, politicians, members of the public "to confront scientists with the implications of their researches", teachers and journalists. An unfortunate omission is mention of those very scientists and technologists whose work raises so many issues. And at the first meeting there was also a sad lack of those same scientists and technologists.

Mr Benn's not entirely unpredictable line was that the questions raised by developments in science and technology were too important to be the subject of closed discussion amongst an elite. He drew two examples, from the military world and the field of nuclear power. The disciplined hierarchy of the military, comparable with monastic orders, could have all sorts of influences of which the public were totally unaware; enormous expenditures in what was now a highly technological defence force were essentially secret. Again, nuclear power, regardless of the merits of those who have devoted their careers to it, raises the some problems of democratic control and tightfistedness over the flow of information. Mr Benn plans to postpone a decision on the commercial fast-breeder reactor for as long as possible, not because of doubts on the technological side but because, he claims, he is as yet not satisfied that the democratic machinery exists to bring out all relevant information.

One of the prices of high technology and high living standards, he pointed out, is a greater vulnerability of society to industrial disputes, technological and natural disasters and war. And as a consequence civil liberties can be eroded—we might find ourselves backing into a police state by means of high technology, and abandoning our values. We cannot, declared Mr Benn, discuss technology in isolation from the much neglected subject of societal values. And if we do not safeguard these values with democratic institutions we are in danger of trading liberties for colour television sets.

Mr Benn, of course, has great faith in the ballot box, in parliament and in fully informed ministers (not as managers so much as members of the public elected as it were to the board of directors). If the public, through elections, can have a voice in the country's social and economic policy, why not also in policy for science and technology?

It is possible to go a long way with Mr Benn's thinking on accountability, and yet to find the ballot box too facile a symbol of that accountability. As he himself admits, some do not vote for the government they believe to be most suitable but (for example) for the candidate who is for or against fluoridation. In all the complexity of motives for voting, and in all the manoeuvring a government does to try and ensure that it is re-elected, what hope that a stable policy for science and technology be central to anyone's thoughts? Yet without stability and rational policies, scientists and technologists who, more than almost any other sector of the community, can move in international circles will simply get up and go where the climate is more settled. And the idea of complex developments with necessarily vast ranges of uncertainty becoming an area of populist concern is bound to strike terror in the hearts of those patiently and quietly trying to accumulate the relevant knowledge.

But whilst we ought to be wary of a sort of free-ranging democracy which is alien to science itself, if the obverse is a return to monastic secrecy, that too must be resisted. Certainly scientists and technologists are a very different breed from politicians, and distrust is to be found on both sides of the fence. But somehow we as scientists have to raise our deplorably low political awareness just as we have to hope that more than a handful of politicians like Mr Benn will find they can move freely and happily within the world of science and technology. Here the Science, Technology and Society Association should have a major role to play. One of its objectives should be to incorporate into the educational system more courses which will make potential scientists and technologists think about public issues. At present it is too easy to become fully qualified without such a training. If this were to make for wiser, more mature judgement by us, the practitioners, and if at the same time an increased number of politicians were able to appreciate the issues in more than terms of short term gain, then science and technology policy, founded on rational dialogue, would benefit immeasurably. And that, presumably, is what Mr Benn would wish. □



Doctors claim A-bomb tests linked to rise in child leukaemia deaths

David Dickson reports on a statistical survey in Southern Utah

RESEARCH workers at the University of Utah claim to have discovered a two-and-a-half fold increase in the leukaemia death rate among children exposed to fall-out from atomic bomb tests carried out in the Nevada desert in the 1950s.

In one of the first systematic studies of civilians exposed to fall-out from the tests, the research workers have found a particularly large increase in the number of leukaemia deaths in children born around 1951—the beginning of the testing period—and that leukaemia death rates returned to their previous levels after the testing was stopped in the early 1960s.

The results of the study have already been passed to federal officials in Washington in a series of meetings last month, but have not yet been published. And although the research workers are cautious about interpreting a correlation as indicating a causal relationship the results of the study will provide a major boost to over 100 cancer patients and relatives of those who have died from cancer in areas surrounding the test site, who are currently suing the US government for a total of \$232 million in damages.

"This is the bombshell that is going to establish beyond doubt that these claims are on solid ground," Mr Stewart Udalla, a former Secretary of the Interior, who is now in private law practice and is pressing the case against the government, said last week.

Residents of Southern Utah have for years suspected that an apparently abnormal level in the number of cancers in the area was linked to fall-out from the A-bomb tests. The two towns of Parowan and Paragonah, for example, with a combined population of 1,800, experienced four cases of leukaemia from 1956 to 1967, two to three times the expected rate.

So far, however, evidence has remained sketchy and largely anecdotal, with insufficient data to establish any significant correlation. Two weeks ago, for example, it was learned that a study carried out in 1965 by a scientist with the US Public Health Service's Division of Radiological Health, who claimed to have shown an excessive level of leukaemia among residents of two Utah counties exposed to fall-out, was ignored and subsequently forgotten.

Interest in a possible correlation between the tests and civilian deaths was revived last year, when evidence was presented to a congressional sub-

committee that an excessive incidence of leukaemia had occurred among soldiers who took part in exercises while the tests were underway (a claim which has recently been substantiated by the Center for Disease Control).

Such evidence prompted the University of Utah's research team, headed by Dr Joseph L. Lyon, of the Department of Family and Community Medicine in the College of Medicine, to carry out a detailed study of child deaths in the state between 1944 and 1975. The results of their study are about to be published in *The New England Journal of Medicine*.

Dr Lyon and his colleagues focused their attention on children under 15 who died in 17 counties in the south of the state, which experienced relatively high fall-out from 26 tests between 1951 and 1958. Radioactive debris from the tests was carried eastwards by the wind from the Nevada test site into Utah.

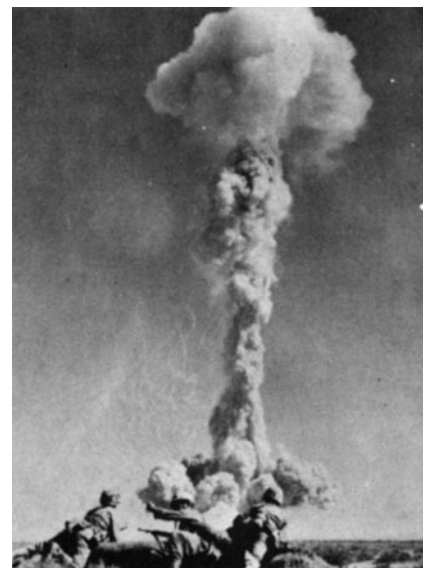
The children were divided into two groups for comparison: a "high exposure" group, who died 1951–58; and a "low exposure" group who died before 1951 or were born after 1958.

Analysis of the death certificates showed the leukaemia mortality rate among children in the low exposure group in these counties to be about half that in the remainder of the state, and indeed the rest of the US—a fact which is unexplained, but may be related to the ascetic life-style of the Mormon communities which predominate in the area.

In the high exposure group, however, although leukaemia mortality started off equally low, it climbed through the 1950s to reach a peak slightly higher than the US average in the early 1960s, subsequently falling back towards its previous level. This group did not reveal any excess for other childhood cancers.

The greatest excess in leukaemia deaths occurred in the 10–14 age group, where the number of deaths rose from between one and two per 100,000 to almost 10 per 100,000 around 1961—implying a particularly high incidence of leukaemia among those born towards the beginning of the testing period, with the disease causing death 10 to 14 years later.

The research workers found that there was a slightly lesser elevation in the 5–9 age group—those who were born around 1956—and little excess among the 0–4 age group. Furthermore the standard mortality ratio for the



Nevada bomb test, 1952, watched by US marines

two groups for the whole age range was slightly higher for boys than for girls.

Even the children who lived in counties which had not experienced the high fall-out rates showed a slight increase in leukaemia mortality. But over half the excess deaths (16 out of 30) occurred in those counties which had been subject to the heaviest fall-out, even though these contained only 10% of the population.

Dr Lyon and his colleagues are being cautious about their results. They do not claim to have demonstrated that the excess leukaemia deaths were in fact due to the bomb tests, merely that there is a statistical correlation between the two. Their evidence is already being studied by other statisticians.

However, enough data has been produced by the study to convince others that when the Atomic Energy Commission told Utah residents in the early 1950s that the fall-out they were receiving was, to quote public statements, "far from hazardous" and presented "no danger" it was being grossly misleading—even admitting that the statements may have reflected the state of knowledge at the time.

Prompted by results such as those contained in the new study, President Carter has directed Mr Joseph Califano, Secretary of the Department of Health, Education and Welfare, to re-evaluate the findings of all earlier studies of the incidence of leukaemia in Utah, particularly in the south-west.

DHEW is expected to name the University of Utah researchers as the principal investigator in a major follow-up study. But whatever emerges, it may be too late to prevent what Mr Udall has called the "terrible tragedy" that took place 20 years ago.

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Budget cuts cause upheavals in earthquake research

David Dickson describes how a sociologist's misbehaviour has led to a major gap in efforts to restrict the damage caused by earthquakes

TWO YEARS ago, Los Angeles City Council member Howard Jarvis—later to achieve fame as author of California's tax-cutting Proposition 13—led a successful campaign on behalf of the city's apartment owners to prevent signs being posted on private buildings liable to collapse during an earthquake. Some months later, sociologists found in a survey that over 90% of the inhabitants of the city, where more than 20,000 buildings are considered to be inadequately protected against earthquakes, thought that the posting of such signs would be a good idea.

Professor Ralph Turner of the University of California in Los Angeles, who heads the research team which conducted the survey as part of a wider project, claims that if the results had been known previously, the city council might have acted differently, and that they illustrate the type of role that the social sciences could play in mitigating the damage caused by earthquakes.

Yet, as the result of congressional actions last summer, National Science Foundation funds for social science research in this area have been savagely cut back. The foundation has been forced to reject all proposals for new projects so that existing ones can be completed. And even in the next fiscal year, funds for such policy research, according to the budget request now being submitted to Congress, will only be one-fifth of the amount originally requested for 1979.

Two years ago, the picture looked very different. With growing concern about a (supposedly) imminent earthquake in California, President Ford requested in his last budget proposal that spending on earthquake hazard reduction programmes at the NSF be

increased from \$12 million in 1977 to \$32 million in 1979 (with an even larger increase at the US geological survey). Last year President Carter responded to legislation passed by Congress by setting out a national earthquakes hazards reduction programme. This stated that earthquakes posed "perhaps the greatest single-event natural hazards faced by the nation" (estimates put the property at risk at \$2,300,000 million), and assigned the NSF to carry out basic and applied research on earthquake engineering and policy.

Then along came Senator William Proxmire. During hearings on the NSF budget, it emerged that a professor of sociology at the university of Colorado, Dr J. Eugene Haas, who had received NSF contracts for more than \$500,000 to carry out research into the socio-economic and political consequences of earthquake prediction, had used the money for, among other things, taking his girl friend on a trip to Honolulu, Tokyo and Hong Kong.

Congress is seldom kind to social scientists, and this misbehaviour, for which Professor Haas was subsequently dismissed from the university, was sufficient for the House Appropriations Committee to reduce the NSF's request for earthquake hazard research from \$26.4 million to \$17.4 million. Within this total, the amount to be allocated to social science policy research was reduced from \$4.8 million to \$800,000, the remainder being divided between siting and engineering design studies.

NSF officials were accused by the appropriations committee of mismanaging the funds awarded to Professor Haas. They were also stung by the claim that similar work was being carried out in other agencies, since

they say these agencies turn to the foundation for their research support. Nevertheless the result has been that the NSF has had to reject all new proposals for research on the policy aspects of earthquake mitigation.

These projects involved assessing the reaction on social and political institutions of information about earthquakes, as well as of the earthquakes themselves. "It is not just a question of needing more research. This is a subject that has never been adequately developed because there has never been a long-term commitment to provide support", Dr Robert Olsen, a social scientist of the Seismic Safety Commission in Sacramento, California, who has chaired a panel on the potential contribution of the social sciences, said last week.

"Many of our problems in reducing earthquake damage relate to legal and institutional considerations that affect the use of technical knowledge and its translation into public policy. The budget reductions mean that the lack of sufficient high quality people willing to undertake such projects is likely to remain."

Dr Olsen's brother, and associate professor in political science at the University of Redlands outside Los Angeles, is one of those who have proposed a major study to the NSF for which he has been told there is no money. The project would involve a two-year investigation in the San Bernardino area of how seismic safety issues are perceived by decision makers. "We shall be putting the proposal in again, and if it flies, it flies. Otherwise this is the last shot. Personally, the lack of money is driving me back into more traditional areas of social science."

Some feel that, despite the tough budget year, the NSF could be more aggressive in its approach to Congress. At present the foundation is requesting a level of funding for 1980 little higher than that to which it was reduced in the current year. "We will do what we can to get this raised, but it would have been easier if they had asked for a little more", the aide to one Californian congressman said last week.

Others feel it may take a major earthquake before research money starts flowing in. According to Dr Carl Kisslinger, president of the American Geophysical Union, the mayor of Pinotep Nacional in Mexico has claimed that public reaction to an earthquake forecast by US scientists did more damage to the economy of his town than the 7.5 magnitude shock that hit the area last August. □



After an earthquake: devastation on the sidewalk of a southern California town.

US scientists want 40% cut in defence spending

A GROUP of six scientists and military analysts has produced an "alternative military policy" for the United States which, it says, would buy a "better and safer defence" at only 60% of the cost of the present policy.

The Boston study group outlines its new strategy to reduce military spending in a 350-page book. *The Price of Defense*, which is being published shortly after President Carter has sent Congress a budget request for 1980 for a 3% real increase in defence spending.

The group says that the study is meant to "stimulate broad and serious debate of overall American military needs and foreign policy"—a debate which it claims has not taken place in the United States for many years.

Speaking at a press conference last week, Dr Martin Moore-Ede, professor of physiology at Harvard University, explained that the Boston group's proposed reduction of 40%—or \$50 billion a year—in American defence expenditure would cut out "inertia, obsolescence and temptation: the inertia accounts for the entrenchment

of high military budgets during peacetime, the obsolescence we cut are the easily-targeted, expensive and vulnerable remnants of the technological past, and the temptation we remove is the excess weaponry and forces that this nation possesses."

More specifically, the study recommends eliminating all of the United States' one thousand land-based inter-continental ballistic missiles over five years, although it concedes that a hundred of the latest Minuteman III ICBMs might be retained longer "as a hedge." All strategic nuclear bombers would also be retired, perhaps over eight years, on the grounds that they are "vulnerable, expensive and obsolete."

The Boston group argues that the retention of 31 Poseidon submarines, each carrying a maximum of 16 missiles, would leave the US with a virtually invulnerable nuclear deterrent sufficient to "wreck every Soviet and allied city and industrial district, plus many extra military targets". It sees no need for the new series of Trident submarines, the first two of which are

under construction, and proposes cancelling them.

The Department of Defense's research and development activities would be severely pruned. The Pentagon would be left with only \$100 million a year to spend on basic research: the group proposes to transfer the remaining \$300 million to a new national technology foundation to "stimulate R & D in areas of expensive high technology where private industry is not yet willing to undertake the work". Applied research and development would be cut by two thirds.

The Boston group analysed the social effects of transferring \$50 billion from the military to the civilian sector of the economy, and estimates that the net effect would be a gain of two million jobs "because defense dollars create far fewer jobs than monies spent in other sectors of the economy."

Clive Cookson

The Price of Defense. Times Books, New York, at \$15.00. Clive Cookson is with The Times Higher Education Supplement.

UNCSTD meeting split on transfer of technology

DESPITE considerable opposition, the transfer of technology to developing countries will occupy a prominent, if controversial, position on the agenda of the United Nations Conference on Science and Technology for Development, in Vienna next August.

This emerged from the third meeting of UNCSTD's preparatory committee, which ended in New York on Monday, and at which delegates from a number of developed countries—in particular the United Kingdom, the US and West Germany—had argued that the issues involved were already being adequately debated in other forums.

In an address to the meeting, for example the British delegate, Dr Dennis Osborne said that attempts to duplicate negotiations on a code of conduct for the transfer of technology already underway elsewhere in the UN system "would lead to confusion and delay in reaching agreement".

Many of the recommendations in the UNCSTD secretariat's preliminary draft programme of action on this topic were "unacceptable to my government in their present form," Dr Osborne said. However Mr Frank Joao da Costa, Secretary General of the conference, told the preparatory committee that the proposed agenda for Vienna and the draft programme merely reflected the wishes expressed by member countries—and that since the transfer of technology was high

among the concerns of many developing countries in their national and regional papers, it would have to be addressed by the conference.

In its closing plenary session on Monday morning, postponed from last Friday to allow additional time for discussion, the committee accepted a resolution submitted by the Tunisian delegate on behalf of the Group of 77 to restructure the draft programmes of action under three 'target areas': reinforcement of the scientific and technological capacities of developing countries; restructuring the conditions of access to scientific and technological

knowledge as an integral part of the new international economic order; and better coordination of the scientific and technological activities undertaken within the UN system.

Despite agreement on the formal changes which they wish to see in the programmes of action, however, the Group of 77, which had established a working party during the committee meeting, were unable to come to any agreement on the substantive issues which the preliminary draft of the programme has raised.

Members of the group, to which over 120 developing countries now belong, have therefore agreed to hold a further meeting before the next preparatory committee meeting, which takes place in May, and to create a mechanism for continuing discussion of the issues up to UNCSTD itself.

According to some observers, it was this strategy on the part of the developing countries which led to the relative success of last year's UN conference on technological cooperation between developing countries. "If this is the case, then the game will have been worth a candle" one of them said.

Delegates at the meeting were unable to agree on whether there should be a fifth meeting of the preparatory committee before UNCSTD, although if such a meeting is called 26 June–6 July has been put aside for it in New York.

David Dickson



"If they delay long enough, the technology they give us will be out of date"

West Germany boosts research by one fifth

Germany is playing for a big lead in innovation, writes Robert Walgate from Bonn

ALMOST without debate, the *Bundestag* last month increased the budget of the West German Ministry for Research and Technology (BMFT) by 19.8% to DM5.5 billion. The emphasis is on stimulating innovation, not basic science. For example, the Deutsche Forschungsgemeinschaft (DFG), responsible for basic research in the universities, received only a 4% increase. But the ministry's programme for stimulating innovation jumped 129%.

There were also big increases in many of the directed programmes of the BMFT, particularly in electronics (32%), transport (58%), and energy, which rises 21% from DM1,720 million in 1978 to DM2,080 million in 1979. The non-nuclear research programme is growing particularly fast. It will reach DM533 million in 1979, 52% above its cost in 1978.

The increase in the transport budget represents confidence in the Erlangen Research Centre in Bavaria, where the government has already invested DM250 million in the Siemens, AEG, and Brown Boveri 450 km/h hovertrain. The investment in electronics will stimulate research in micro-processor technology.

The BMFT was not the only ministry to increase its research budget: the Ministry for Industry also announced a DM300 million boost for research in small and medium sized firms, to cover up to 30% of the cost of employing new research staff. It is hoped that this will not only correct the imbalance whereby some 90% of government-supported industrial research has been allocated to only 40 firms, but might also provide employment for the 50,000 or so un- or under-employed scientists in Germany.

Germany may be in a position of great economic strength at present, but it is certainly taking very seriously the challenges provided by its lack of natural resources, and the increasing industrial competition from Third World countries with their low labour costs. Germany seems determined to invest effectively in its brains. However there may be a more immediate reason for the success of the BMFT in this budget. The previous minister, Hans Matthöfer, is now Finance Minister in overall control of the budget, so Volker Hauff, the 38-year-old economist who now runs BMFT, has had a sympathetic ear.

However, the *Bundestag* itself showed little interest in the developments. Despite a two-day debate (23-24 January) on the whole German Budget,

only the last 90 minutes (from 10.30 pm to midnight) were devoted to the affairs of the BMFT. A leading commentator on science politics in West Germany, Hartmut Altenmueller, wrote: "The interest that politicians and the public take in science was shown by the debate in the *Bundestag* last week. Zero." Only one parliamentarian, says Altenmueller, criticised the situation. This was Herr Laermann of the Centre Party, FDP (which has been taking an interest in ecology and the Kalkar fast breeder reactor lately). Laermann argued that it was time the *Bundestag* took an interest in the politics of science and technology before the government had formed its policy, made its mistakes, and presented the *Bundestag* with a *fait*



Technology minister Volker Hauff

accompli. But little attention was paid to his speech.

On a longer perspective, however, German science politics is becoming increasingly interesting, and it would seem impossible that the *Bundestag* could ignore the issues. For example, Professor Helmar Krupp, Director of the Institut für Systemtechnik und Innovationsforschung (ISI), believes that there is a growing awareness in Germany that technology must be looked at through the eyes of labour: what is it like to work with technology, what influence does it have on life? Moreover, this concern, he feels, is not the exclusive property of the ruling SPD coalition. "The awareness is greater here in Germany that we must rely on high technology, that intensive work with raw materials will go out".

The government is increasingly turning to institutes like his own (it is similar to the Science Policy Research Unit at the University of Sussex in the

UK) to ask for expert advice in matters of science and technology policy. For example, the DM300 million programme for stimulation of research in small and medium sized industry was designed within ISI in close co-operation with the ministry.

The energy issues, says Krupp, are much the same in Germany as elsewhere. But in basic science, Germany is facing some interesting questions. The Max Planck Institute for Plasma Physics in Garching, near Munich, is recovering rapidly from the blow of losing the joint European Tokamak (JET) project to Culham in the UK. It is now forming a proposal for a device not dissimilar in price to JET itself.

JET was delayed for some years by Brussels politics, and in some respects its design is dated. The Garching project, costing DM280 million, would be designed specifically to achieve nuclear ignition in the plasma, whereas JET is primarily a device to test scale-up (though it may also achieve ignition). The Garching device would be designed to take account of the radiation hazards.

The proposal is not yet finalised even within Garching, because one of the divisions (high beta plasmas) would have to close. But it is the main contender for Garching's main programme after ASDEX (a device under construction to test the concept of a 'divertor' to clean impurities from the edge of the plasma column). The ignition device would probably be a collaboration between Garching and MIT in the US. The view of the BMFT is not known, but, says a physicist at Garching, "at least they haven't stopped the study".

Another issue which seems sure to come to a head soon lies in a conflict between the basic research funding body DFG and the ministries concerned with a government special programme on 'health research'. Officials at the DFG are worried that criteria used for selecting research for funding under the independent, DM450 million five-year health research programme are too lax. "There is a problem of inflation of poor projects", said one DFG official. "Projects are defined loosely, and so are the assessments". Furthermore the ministries reserve the right to ignore the advice of the scientists they appoint to do the assessments, so that poor research can be funded purely on political grounds.

More recently the DFG has been asked to set up a committee to advise the government on cancer research. It may well be in that committee that the issue will be raised. □

US at sixes and sevens over Soviet exchanges

LAST week, Dr Frank Press, the US presidential adviser on science, left for Moscow to discuss the progress of US-Soviet scientific cooperation.

The visit, as part of the regular programme of scientific cooperation inaugurated by the Nixon-Brezhnev agreement of 1974, was originally scheduled for last summer. It was cancelled at the last minute by President Carter in protest at the trial and conviction of Dr Yurii Orlov, the physicist, for human rights activities.

The Soviet science planners clearly welcome the restoration of full scientific relations with the US. Writing in *Izvestiya*, Dr Gvishiani, Deputy Chairman of the State Committee for Science and Technology stressed the "mutual benefit" of such exchanges and answered Western attempts to impede them. He maintained that technology transfer was not as is frequently alleged, a "one way flow"; several US firms, he said, were benefiting from Soviet technology.

At present, except for a few hard-liners, there is little support among Western scientists for out-and-out boycott of the Soviet Union. Most would prefer to avail themselves of the opportunities provided by conferences in the Soviet Union to visit those of their colleagues who are dissidents and refusniks.

During 1978, however, some US academics claimed that the State Department and other relevant departments and agencies of the US government appeared to be implementing a policy of discouraging US scientists, who were visiting the Soviet Union as part of the official exchange programme, from contacting dissidents and refusniks.

It appears that pressure ranged from "friendly warnings" to the dismissal of certain known friends of the Soviet "opposition" from honorary but prestigious US government consultancies.

At the end of last year, the Committee for Concerned Scientists Inc., one of the most active human rights pressure groups, made representations to the State Department that this practice should cease. The committee was assured that no such policy in fact existed; if any such actions had been taken, it was on the mistaken initiative of individuals.

One result of the confusion of official US policy on the matter is that, on occasion, the Soviet hosts have been able to exert pressure on visiting scientists to stay away from opposition circles. In one case, a party of scientists was informed by the head of the Soviet welcoming committee that he had an "agreement" with the head of the US exchange programme in physics that scientists on official exchanges should not visit the Sunday seminar for refusniks. Challenged on the matter, the US official in question denied the existence of any such "agreement", as did also the National Academy of Sciences and the various government agencies in charge of exchanges.

If the idea of general boycott is abandoned, scientists concerned about human rights issues could take action by "working to rule" on the vexed question of substitutes. For many years Soviet delegates invited to attend conferences outside the USSR have been replaced by scientific nonentities. Originally, this resulted in free periods in the time-table, but gradually over the years it has become increasingly common for the substitutes to be accommodated in the programme.

Last summer, at the Paris conference on luminescence, a special "Round Table" on the Orlov case was held, and the possibility of a campaign against substitutes discussed. The consensus of opinion, however, was that it would cause too many practical difficulties, and possibly lead to Soviet withdrawals.

So far, only a few hard-liners, such

as Nobel Laureate Paul Flery and his four co-protesters, who refused to attend the IFPAC seminar on Macromolecular chemistry in Tashkent, are prepared to make a stand on the "substitutes" issue.

However, a disturbing document issued recently by the Committee of Concerned Scientists may well make it necessary to reappraise the whole problem of substitutes. The report, alleging wide-spread anti-Semitism in Soviet mathematics, stresses that it is virtually impossible for Soviet Jewish mathematicians to travel abroad to conferences. This, it must be stressed, is not a matter of Jews who wish to emigrate to Israel, but simply of Soviet citizens with no "Zionist" inclinations who have a Jewish name and the entry "Jew" on their internal passport.

The Committee for Concerned Scientists strongly urge a "no-substitutes" policy as the best way of helping in such cases. Other countries of the Comecon bloc are, in the main, somewhat more lenient about foreign travel—with the notable exception of conferences held in Israel, which, since 1967, has had no diplomatic relations with any Comecon country except Romania. Poland is perhaps the most lenient of the Comecon countries. A handful of Polish ecologists managed to attend the Intecol-2 conference in Jerusalem last September. However, members of the Polish Biochemical Society are not prepared to rely on this precedent. Already they are making known abroad their wish to attend the meeting of the Federation of European Biochemical Societies in Jerusalem in August, 1980.

It is not sufficient, they stress, that one or two leading Academicians should attend as official delegates—what they are seeking is that any scientist from the Comecon bloc, willing to travel at his own expense, should be permitted to do so.

Vera Rich

Moscow's growing pollution problem

INDUSTRIAL pollution in the Moscow region will increase by 150-200% by the end of the century, according to Dr Aleksandr M. Ryabchikov of Moscow State University. Already the city has spread across the ring road originally intended to confine it, and new developments are penetrating into the 'green belt' economy—dairy farming, market gardening, leisure zones and reservoirs, of what is described as the 'second peripheral belt'.

Purification installations are compulsory for all new industrial enterprises,

but, according to Dr Ryabchikov, are "insufficiently advanced" and are very expensive, costing up to 40% of the capital cost of the plants. Only the use of abundant non-polluting energy—thermonuclear fusion and solar energy based on photochemistry, he said, can provide a lasting solution to the problem.

Dr Ryabchikov's suggestions follow a broad-based research programme on environmental measures recently been carried out in the geographical faculty at Moscow University.



Moscow: Time for a clean-up

Sigvard Eklund: the uses and abuses of nuclear energy



● The director-general of the IAEA (above) put the pro-nuclear case in London last week. Judy Redfearn reports

THE increased use of nuclear power has "not contributed to horizontal proliferation and is unlikely to", according to Dr Sigvard Eklund, director-general of the International Atomic Energy Agency (IAEA). At the presidential lecture of the British Institution of Nuclear Engineers in London last week, he supported this claim by saying that only six states have exploded nuclear weapons although many more have the technical know-how to do so.

The political will of states is the key to controlling proliferation, according to Eklund. Without it, international safeguards cannot be effective and "technical examinations are valueless".

Restricting the supply of enriched uranium and nuclear technology to developing countries, as advocated by the United States, is only likely to "encourage nuclear self-sufficiency and therefore increase proliferation", Eklund claims. He suggested the IAEA could work out a scheme to assure countries of essential nuclear supplies. This would have the strength of current non-proliferation arrangements and would assure supplies and safeguards. A disruption in supplies would be a breach of the safeguards. There was a need for urgent action, he said. "Nations would gain more in partnership than in imposing their thoughts on others."

Predictably Eklund is a staunch nuclear supporter. The nostalgia he feels for the time when nuclear engineers were left alone to solve difficult problems "quickly" and impossible ones "with time" illustrates his attitude towards the arguments of the anti-nuclear movement. "Rational arguments", he says, "no longer carry any weight."

Eklund believes that nuclear power will be needed to meet increasing world energy demand. He is sceptical of energy forecasting however: the great variety of estimates of future energy demand are not surprising, he says,

considering the many different assumptions energy forecasters have made.

Nevertheless, he believes that the growth in demand will continue over the next decade (though the rate of growth will fall). Even if the developed countries use conservation measures, he says the world demand will continue because of increasing population, economic development, the development of sparsely populated areas (such as Siberia) and the energy needed to recycle increasingly scarce natural resources. "Even if the developed countries have zero energy growth, demand from developing countries will lead to a substantial growth in world energy needs."

Moreover, he says, conservation takes too long to have an effect to be a major factor in reducing energy demand before the end of the century. "Lifestyles and social structures don't change overnight, at least in democratic systems". Neither does he foresee any major role for alternative sources of energy. Those with the greatest potential such as hydroelectric power, he says, are already being exploited and others probably won't have much to offer.

So, in the expectation of increasing demand, Eklund sees nuclear power as ripe for a substantial contribution—at the right stage of development and economically competitive with other energy sources. "I don't believe uranium prices will rise faster than oil prices", he says, and substantial new discoveries of fossil fuels could only postpone the need for nuclear by a few decades. Oil is likely to become increasingly scarce and costly and a rapid expansion in coal production would lead to "serious environmental problems."

Eklund puts a lot of emphasis on fast breeder reactors which, he says, could double the energy potential of uranium and provide the world with abundant energy. Since 1973, however, there has been a drop in the number of FBR projects. "This constitutes a paradox", says Eklund. "Nuclear power has become the victim of the pioneering work on the environment done by its founders." The anti-nuclear movement has become allied to political groups which search for a better 'quality of life'—a term never defined, according to Eklund—rather than a higher 'standard of living'. These groups are not found in totalitarian states, he says: their greatest influence has been in countries with low majority governments such as Sweden and Austria. □

Can multinationals aid development?

A RAPID technological transformation, similar to the industrial expansion of Japan in post-war decades, is now facing several developing countries scattered round the globe, according to Dr Jorge Katz, director of the Economic Commission for Latin America's research project on technology and development. Indeed, these nations—which include Brazil, Argentina, Mexico, South Korea, Singapore, and perhaps even India—may soon be in a position to export advanced technical programmes to other countries—although their spread will be on more limited industrial fronts than Japan's.

The slower-developing nations of Central and South America, and also Africa, then become the main targets for industrial and technological exports from their more advanced neighbours. The question then is what these less sophisticated nations will gain in the transaction.

It is generally assumed that developing countries which are used as the sites for the advanced industrial complexes of multinational corporations gain little in the way of technological knowledge of expertise. But Dr Katz challenged this view at a recent symposium at Sussex University, organised by the Science Policy Research Unit there. He maintained that developing nations were able to assimilate information from sophisticated manufacturing plants in their midst.

"You cannot take a piece of technology from a developed country, put it in a developing country and expect it to work in exactly the same way", he said. Altered circumstances often led to changes in plant design and in this way, local engineers and technologists became involved in work that increased their expertise.

However, a variety of economic factors affected the nature of technological transfer gained at plant level. One important determinant was the amount of freedom given by multinational companies to their foreign subsidiaries. For instance, many firms in the United States allowed their subsidiaries to vary original designs, while European, especially Swiss and German, companies tended to forbid this.

Other determinants included the rate of capital subsidy; the rate of exchange; and the cost and variability of local skilled labour. In some parts of Africa, there was practically no skilled labour available. This handicapped the setting up of new factories and in turn prevented new expertise being generated.

Robin McKie

news in brief

WHO endorses world wide barefoot doctor campaign: The Executive Board of the World Health Organisation has approved a plan for radically improving health care throughout the world by the year 2000. Described as "a landmark in public health history" the plan addresses the failures of present medical establishments to provide satisfactory health care for the great majority of people and proposes the use of appropriate technology, improved nutrition, increased attention to water supplies and the greater use of health auxiliaries, the barefoot doctors pioneered in China.

The WHO proposals stress self reliance and technical cooperation between third world countries. According to a WHO spokesperson the plan calls for a shift away from dependence on transnational drug companies—at present countries spend up to one half of their health budgets on "useless" drugs—and away from reliance on "the great white experts" from Europe and the United States. The board also stressed that good health cannot be separated from economic and social development.

Joint UK-US reactor safety research planned:

A joint testing programme on the safety of plutonium fuel will be signed shortly by the UK and the US. The programme will make use of the UK's 250 MW prototype fast reactor at Dounreay and the United States transient reactor test facility (TREAT) located at Idaho Falls, Idaho. Plutonium fuel pins will be burned at Dounreay for varying periods up to their lifetime of two years. After a cooling off period, the pins will be transferred to the TREAT facility where, singly and in bundles, they will be subjected to rapid excess reactivity. This procedure generates rapid temperature changes in the pins and is a way to simulate loss of coolant or control rod malfunction accidents. The pins can then be analysed for structural soundness against this type of accident.



The magazine, *Undercurrents* recently reported that water was found coming out of a 4 inch crack in a primary cooling pipe at the Duane Arnold nuclear reactor in Palo, Iowa. Upon inspection all seven of the cooling pipes were found to be cracked. Two plants of similar design in North Carolina have been shut down for inspection and at least nine others are at risk. A complete break in one of these pipes would cause a major loss of coolant accident of the type that the TREAT facility means to test.

US Congress elects liberals to key science policy committee posts: In a major upset to the traditional seniority system, the US democratic members of Congress have elected liberal candidates to two key subcommittee posts on scientific and environmental issues over the heads of more senior contenders.

Representative Henry Waxman of California, who was only elected to Congress in 1974, has been chosen to succeed Representative Paul Rogers as chairman of the Health and Environment Subcommittee of the House Interstate and Foreign Commerce Committee.

This committee has been the source of much recent health research legislation, as well as of concerted attempts to produce legislation covering recombinant DNA research. Mr Waxman defeated the more senior representative Richardson Preyer, who had aroused certain suspicion through holding financial interests in various pharmaceutical companies, an area of the subcommittee's responsibility.

A further surprise was caused by representative Toby

Moffett, another post-Watergate congressman, who was elected to chair the Subcommittee on Environment, Energy and Natural Resources of the House Government Operations Committee.

The two appointments represent victories for labour, consumer and environmentalist interests. However, with a new generation of congressmen arriving in Washington bearing a considerably less liberal outlook than some of their immediate predecessors, few are expecting any radical shift in the activities of either subcommittee.

Short term contract researchers meet in Manchester:

Research workers from the University of Salford, Manchester University and the University of Manchester Institute of Science and Technology met on 31 January to discuss problems of short term contract workers. Participants discussed individual cases, including that of David Pillinger (25 January, page 255) a tenured cancer researcher made redundant at the Christie Hospital: present deficiencies in the conditions of service of short term contract workers; and long term prospects including a proper career structure and strategies for long term funding including the possibility of prying money out of the University Grants Committee to fund research workers instead of just equipment and buildings as is presently the case. According to convenors Jean Shaoul and Alastair Risk of the University of Salford the questions raised are "fairly standard union matters" but the easy money of the 1960s has spoiled researchers with the result that "present organisation is well behind the rest of the working population." The next meeting is scheduled for 7 March at Manchester University.

Refusnik resists move into secret work: Since he applied to emigrate to Israel in 1974, the position of Professor Solomon Alber, a topologist working at the Institute of Chemical Physics of the Soviet Academy of Sciences has been anomalous. Normally, any such applicant is immediately dismissed from his job; Alber, however, was simply demoted from head of the mathematics department of the institute to a minor academic post. Recently, attempts have been made to shift him to the branch of the institute doing secret work—a ploy which would effectively obviate his chance of ever receiving a visa. Alber is resisting the move. If, however, he fails to comply, the institute will have some basis for his dismissal other than that of his wish to emigrate.

GE researchers develop plan for chemical heat pipe: A research team at General Electric's Research and Development Centre in Schenectady, New York has produced a feasibility study for using industrial waste heat. The study is based on an original German proposal in which heat is used to drive the catalytic conversion (at 1900 °F) of methane to carbon monoxide and hydrogen, $\text{CH}_4 + \text{H}_2\text{O} \rightarrow \text{CO} + 3\text{H}_2$. The reaction products are then cooled (the heat being used to warm incoming methane) and are piped over distances up to 300 miles where they are re-combined catalytically in the exothermic reverse reaction, the heat being used locally.

GE scientists sought to find a reaction that would go at the lower temperature of 1000°–1200 °F generated in many types of power station. They found a candidate in the catalytic conversion of cyclohexane to benzene and hydrogen, $\text{C}_6\text{H}_{12} \rightarrow \text{C}_6\text{H}_6 + 3\text{H}_2$. The efficiency of the heat conversion and extraction is estimated to be 80% but there are undetermined particle losses in the catalytic reaction. A loss of 1% per cycle would make the proposal unworkable. Research is proceeding to determine this factor.

A modest contribution to security and cooperation

TUCKED away among the fifty pages of the Helsinki Agreement is a short paragraph envisaging a 'scientific forum'—"a meeting of leading personalities in science from the participating states". In the two years after Helsinki, this item seems to have been given little attention. But amidst the wrangling at Belgrade last year, it was a relief to agree to set up an innocuous meeting of experts to plan the forum.

This meeting took place in Bonn, during the summer. For six whole weeks, diplomatic and scientific representatives of the 36 signatories of the Final Act of the Conference on Security and Cooperation in Europe (CSCE—alias the Helsinki 'Agreement')—circled warily and delicately around this modest-seeming proposal. Their eventual report was unanimous: the scientific forum will be held in Hamburg, in February 1980.

From the diffuse agenda proposed in this report it is difficult to estimate the significance of the forum. Two weeks of discussions of "interrelated problems of common interest concerning current and future developments in science" are not likely to throw much new light on the human condition, especially when dispersed over such a diversity of topics as "alternative energy sources", "food production", "trends in medical research, in particular in basic research and primarily on cardiovascular, tumour and virus diseases, taking into consideration the influence of the changing environment on human health", and "comparative studies on social, socio-economic and cultural phenomena, especially the problems of human environment and urban development".

In the diplomatic bargaining that produced this list, it was obviously easier to achieve a consensus by including the favourite topic of every delegation than to spend another six weeks trimming it down to size. Thousands of man-weeks of serious discussion of all these topics is already taking place each year, uninhibitedly across all the frontiers of the world; nobody is under any illusions that the forum can do much more scientifically than skim lightly over very familiar ground.

The original text of the CSCE Final Act intends that the forum should "promote the expansion of contacts, communications and the exchange of information, between scientific institutions and among scientists". But here again, a meeting of this kind can do little to supplement the existing network of bilateral and multilateral agreements, involving national scientific organisations, transnational scientific

An international science forum is to be held in Hamburg a year from now, in the wake of the signing of the Helsinki Agreement (right) John Ziman, professor of physics, Bristol University, discusses his hopes for the meeting.



unions, and international agencies, under which such activities already take place on a very large scale. In this respect, the further implementation of the Helsinki Agreement to improve cultural and scientific contacts depends essentially on the amelioration of the general political climate and on detailed attention to a multitude of small obstacles to free traffic in ideas and people.

Nevertheless, those of us who took part in the Bonn meeting came to appreciate the value of the proposed forum as a symbol of the Helsinki spirit, with a modest potentiality for good, both for the progress of science and for the welfare of the people of Europe. In the acrimonious aftermath of Belgrade, it was natural that the proceedings at Bonn were ponderous, and the agreed agenda turned out to be rather trite. This was a diplomatic meeting, with all the rigmaroles of achieving complete agreement amongst the representatives of sovereign states.

If the scientists had been left alone to plan the forum, they might not have been quite so sensitive to the protocols of arriving at consensual proposals within the interlacing alliances of the 'nine' (the EEC) and the 'fifteen' (NATO). Even where there is sharp conflict of interest, as between the countries of the Soviet bloc and the Western democracies, scientific leaders can usually rely upon the shared experiences and common goals of research, so that negotiations of this kind are seldom conducted at arm's length.

But behind the courteously meandering debates and apparently trivial verbal amendments of text lay real issues, which might well have been compromised in a less self-conscious bargaining process. What is science? Is it the technological arm of state power, or does it transcend national frontiers? Should those 'leading scientific experts' who are to participate so gravely in the forum be cast as official representatives of their respective governments,

or should they appear as individuals supported by the authority of their scholarly achievements? Are the topics to be treated largely from the aspect of practical application—hence within all the constraints of economic, political and military competition—or in those fundamental aspects that are universal and free for all mankind? These questions were a constant preoccupation of the diplomatic and scientific representatives at Bonn, on both sides of the Great Divide.

On the whole, the agenda of the forum leaves the door as open for truth as for the manifestations of power. This is something of an achievement. From the beginning, the Soviet government and its closest allies were fearful that the West might be tempted to use the forum as an instrument of protest against the oppression of dissidents. Their desire to impose official representative status on the speakers at the forum was as much an attempt to muzzle such 'undiplomatic' mouthpieces as a reflection of their own heavily bureaucratised and subservient scientific systems. The Bonn report does not impose such a gag, thus effectively recognising the universality of the voice of science.

In my view, it would be a mistake for Western scientists to press the human rights issue at the forum. This I say reluctantly, for this issue is profound within modern science. But what is offered at Hamburg, in little more than a year's time, is the opportunity to celebrate, under official governmental auspices, as publicly and as explicitly as could be, the genuinely transnational character of the scientific enterprise. It will be an occasion to demonstrate the universality of empirical fact and rational argument, the communal spirit that pervades research work in all fields, the fraternity and cosmopolitanism of the republic of learning, and the humane benefits that accrue from a body of reliable scientific knowledge. These are the norms to which we refer, these are the ideology of science itself—the ideology to which the dissidents themselves appeal in their opposition to tyranny.

The Helsinki Agreement was about cooperation and détente. The true meaning of these unhappily tattered concepts in the scientific and cultural domain must not be forgotten. The value of the scientific forum is unlikely to be found in deeper understanding of technical scientific issues, nor in another confrontation on socio-political fundamentals, but perhaps in a modest affirmation that truth itself is one of the powers to be reckoned with in the real world. □

correspondence

Causes of cancer

SIR,—In a recent book review I stated that "many, and perhaps most, cancers are caused by certain sexual habits, smoking habits, and gross aspects of diet rather than contaminants" (27 July, page 404). My statement runs counter to the general belief that we are currently suffering an epidemic of cancer due to a multitude of carcinogenic pollutants, and I have been asked, both courteously in private correspondence and sarcastically by Ralph Chernoff (7 September, page 8) to substantiate it. Chiefly, those who are surprised by this statement should read the relevant reviews of cancer epidemiology (*Nature* 265, 589; 1978) or of cancer trends, but some relevant points can be summarised briefly.

The major age-specific trends over the past decades are not in general upwards, with the notable exception of those cancers associated with cigarette smoking. The American Cancer Society (*A Journal for Clinicians*, 28, (1), 20–21; 1978) have illustrated this for America, and Doll (*Proc. Roy. Soc.*, in press) has shown it in more detail for Britain, avoiding the older age groups where the progressive elimination of mis-diagnosis produces artefactual trends. Any balanced perspective on cancer must begin with the role of smoking, not with any other aspect of the modern world. (The arguments recently advanced by the office of OSHA in the US that 20–40% of all cancer will soon be caused by industrial exposure are methodologically unsound, for the particular risks which they discuss are very considerably exaggerated.)

Smoking, particularly of cigarettes, is the chief cause of lung cancer in Britain, and smoking is strongly associated with oral and with oesophageal cancer and also, albeit less strongly, with bladder and with pancreatic cancer. Adding up appropriate proportions of the numbers who die of these cancers, smoking is responsible for about 30% of all British cancer deaths, most of this 30% being accounted for by the solidly established causal relationship between smoking and lung cancer.

Sexual habits are the chief known determinant of the risk of cancer of the uterine cervix, a disease which is very much commoner in prostitutes than in nuns and which is commoner among women who have had many sexual partners or who began intercourse at an early age. This disease, however, accounts for only 2% of all British cancer deaths, and although we have accounted for "many" British cancer deaths by smoking, we have not yet accounted for "perhaps most" deaths, and cannot do so unless we can describe likely causes for stomach, breast or colorectal cancer.

Stomach cancer used to be very common in the United States, but over the past forty years the mortality from it has decreased tenfold, so that it is now quite rare. The causes for this are quite obscure, especially since treatment has not materially improved, but since downward trends in stomach cancer are at last also

developing in Britain one can hope that whatever (dietary?) aspects of the twentieth, as opposed to the nineteenth, century environment has protected the Americans will also eventually protect us.

In Britain, apart from lung (27% of cancer deaths) and stomach (9%), the two commonest sites are breast (9%) and large intestine (13%), and preventable causes for these definitely exist although they cannot as yet be identified with certainty. Early pregnancy seems to protect against breast cancer, but no practicable other way of producing these protective effects is yet known, so let us turn to diet instead. Among rats that have been pre-treated with certain carcinogens, a moderate dietary increase or decrease of certain components of "fat" will greatly increase or decrease their chances of developing breast or colon cancer. In humans, graphs of the per caput fat consumption in various countries against age-standardised cancer rates have been found to yield very striking positive correlations with fat for female colon cancer (81% correlation), for male colon cancer (85%) and for female breast cancer (89%). These correlations, taken together with the evidence in laboratory animals, make it plausible that some gross aspect of diet is a preventable (since people already differ with respect to it) cause of these cancers.

Of course, one particular person's fatal cancer may have had more than one preventable cause, especially if multistage models for cancer induction are approximately correct (Peto, 1977, *Origins of Human Cancer*, Cold Spring Harbor publications). However, this makes it more, rather than less, plausible that among "sexual habits, smoking habits and gross aspects of diet" (such as the consumption of certain fats or fibres) we shall find at least one preventable cause for many, and perhaps most, of today's fatal cancers.

Yours faithfully,

RICHARD PETO

Radcliffe Infirmary
Oxford, UK

World futures

SIR,—I would like to point out some curious omissions in Lord Ashby's review of Freeman's and Jahoda's "World Futures" (9 November, page 144).

Ashby cites with approval the choice of four growth options; he forgets that there are also four decay options, which should not be left out *a priori*, merely on the ground that they are less cheerful than the former.

When Ashby agrees with the "outstanding practical conclusion" from the analysis, namely that the absolutely top priority is to narrow the gap between rich and poor, both within and between nations, and that this can only be done by devoting massive resources to certain problems, he fails to mention two important facts. First, that this narrowing of the gap between rich and poor is exactly what has been happening in Western industrialised

countries for the last hundred years; second that the same thing is happening now between nations, without any wilful action at all. Although most poor nations have not become richer, western European countries have become immensely poorer, through the operation of political, economic and social forces, such as displacement of industry, loss of control over resources and the corresponding inflation. If this trend continues, these nations will be fully-fledged members of the Third World within a very short time, and this would of course resolve the problem of their devoting massive resources to aid other countries.

Yours faithfully,

S. V. VAECK

Ministère des Affaires Economique,
Brussels, Belgium.

Energy forecasts

SIR,—Your correspondent reports Amory Lovins commenting favourably on the predictive power of his tabulation of US Energy Demand Forecasts, (18 January, page 163). My analysis supports the opposite view.

Neglecting Steinhart's forecast for 2050 as outside the boundary, and taking instead the lower CONAES 2010 figure as "beyond the pale" for '78, you will discover that the range between maximum and minimum for each year of forecasts is nearly constant at 60 q. Thus as the year of forecast approaches the target year 2000, so the percentage error on the forecasts increases! Is this knowing more and more of less and less?

Yours faithfully,

J. T. D. MITCHELL

Abingdon, Oxfordshire, UK.

ARMS cannot negotiate

SIR,—We were gratified to read Mr Hounsell's letter (18 January, page 171) and his expression of support for ARMS albeit with reservations. Let us allay his fears on one or two points. First, the prime objective of ARMS is to seek to develop and establish career structures for full-time research scientists in biomedical sciences, and who could be better motivated than the scientists themselves to do this. Second, we recognise that we have neither the experience nor the rights to 'negotiate' with employers (in the trade union sense) but we can at least enter into discussions, identify problems and make recommendations to both employers and the unions who aim to represent us. If researchers become diverted from working within a trade union as a result of this, then it can only mean that the unions themselves are failing to fulfil the requirements of these workers.

Yours faithfully,

ARLEEN UNGER

ARMS, Clinical Science Labs.,
Guy's Hospital, London, SE1.

news and views

Synthetic genes for human insulin

from Michael J. Gait

THE synthesis of human insulin by bacterial cells fermented on production scale, a dream of molecular biologists for several years, is rapidly approaching reality with the recent announcement of the expression of synthetic human insulin genes in *E. coli* (Crea *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 5765; 1978; Goedell *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press).

The dream began early this decade with the discovery that pieces of DNA of different origin could be inserted into a bacterial plasmid or virus (*in vitro* recombination). The chimaeric DNA could be used, for example, to transform *E. coli* and clones containing the desired insert selected. It was not difficult to imagine that one day DNA coding for insulin could be inserted into a bacterium and expressed as mature insulin. The idea was also attractive to pharmaceutical industry, conventional methods of insulin production being hopelessly inadequate for projected future needs.

Recently several giant strides have been taken towards insulin expression in bacteria. First, rat pre-proinsulin mRNA has been reverse transcribed into cDNA and inserted into the plasmid pMB9, which was used to transform *E. coli* cells. In one clone the whole proinsulin coding sequence and part of the N-terminal pre-peptide was present (Ullrich *et al. Science*, **196**, 1313; 1977; see also *News and Views* **268**, 198; 1977). More recently Villa-Komaroff *et al. (Proc. natn. Acad. Sci. U.S.A.* **75**, 3727; 1978) inserted cDNA, again made from rat pre-proinsulin mRNA, into the coding sequence for the secreted protein penicillinase located on the plasmid pBR322. After transfection and cloning a fused penicillinase-proinsulin translation product was detected to be secreted from the bacterial cell. Isolation of human pre-proinsulin mRNA is technically difficult and has not yet been accomplished. Fortunately an alternative and poten-

tially much more far-reaching approach is now possible based on the use of chemically synthesised DNA.

As a curtain-raiser K. Itakura *et al. (Science* **198**, 1056; 1977) showed that synthetic DNA corresponding to the sequence of the peptide hormone somatostatin could be inserted into the plasmid pBR322, this time into the gene for the secreted protein, β -galactosidase. An extra methionine codon was incorporated at the N-terminus of the somatostatin gene sequence, so that after expression in *E. coli* the secreted, fused, β -galactosidase-somatostatin product could be cleaved at the methionine residue by treatment with cyanogen bromide, liberating somatostatin (which contains no methionine).

The same research group has now described the chemical synthesis of two pieces of DNA corresponding to A and B chains of human insulin (Crea *et al. op. cit.*). Each DNA duplex was separately inserted into the plasmid pBR322 in the same way as for the somatostatin gene. After transformation of *E. coli*, fused β -galactosidase-insulin A in one case and β -galactosidase-insulin B in the other were found to be secreted (Goedell *et al. op. cit.*). Free A and B chains are generated by cyanogen bromide treatment, but so far only A chain has been isolated in reasonably pure form. Nevertheless, yields of insulin chains are about 10 mg per 24 g wet cells, about 10 times higher than for somatostatin.

The aim is to reconstitute mature insulin by association *in vitro* of disulphide bonds between A and B chains (proinsulin *in vivo*, consisting of B chain, a connecting peptide and A chain, folds and forms two disulphide bonds between chains A and B. The connecting peptide is then excised to generate mature insulin). One drawback to synthesis of separate insulin chains is that the achievement of high efficiency of disulphide bond association in the correct orientation is currently disputed amongst specialists. Goedell *et al. (op. cit.)* indeed report low yields

when impure A and B chains are used and so far have only detected mature insulin by radioimmunoassay. A better approach might be to synthesise the DNA sequence corresponding to pro-insulin or an analogue with an easily excisable connecting peptide. Here disulphide bond formation *in vitro* is likely to be much more efficient because A and B chains are already in close proximity. However, chains of a possibly active human gene in *E. coli* would require much more stringent containment under the present guidelines than the P2-EK2 used to clone the chains separately. The safety of this procedure would need to be established before large scale cloning could be considered.

Despite the uncertainty of insulin reconstitution the methodology as it stands must be recognised as a remarkable contribution to molecular biology, at least as important as the pioneering synthesis of RNA genes by H. G. Khorana's group (see Khorana *et al. J. biol. Chem.* **250**, 565; 1976; the synthesis of the tyrosine suppressor tRNA gene has finally been completed (T. Sekiya *et al. J. biol. Chem.*, in the press)). Khorana introduced the technology for joining chemically synthesised oligodeoxyribonucleotides 10–15 units long with the enzyme T4 DNA ligase. Using the specific base-pairing mechanism two such chains could be joined in the presence of a third 'splint' chain. A duplex is formed by simultaneous joining of several chains aligned in this way. Crea *et al. (op. cit.)* have used the same enzymatic technology, but the chemical synthesis of the 29 oligonucleotides needed to form the two duplexes has taken only a fraction of the time (in man-years) compared with that required for the tyrosine tRNA gene. This is the result of the use of the developing 'phosphotriester' synthetic technology (compared with Khorana's phosphodiester approach) as well as new rapid purification techniques afforded by high performance liquid chromatography.

Now that synthetic DNA can be prepared on a reasonable time-scale the

Gravitational radiation at last?

from P. C. W. Davies

SCIENTISTS have used astronomy as a proving ground for theories of gravity ever since Newton explained the sizes and shapes of the planetary orbits. In 1915 Einstein proposed a new theory of gravity—general relativity—involving bizarre concepts of geometrically distorted space-time, and in a short while minute corrections to Newton's theory were confirmed by careful observation in the Solar System.

Until recently, no qualitatively new tests of general relativity were made. Then, with the discovery of a weird type of double star in which gravitational fields are thousands of times stronger than in the Solar System, the possibility arose of confirming subtle higher-order relativistic corrections to Newtonian gravity. Known as a binary pulsar, the new object is thought to be a pair of imploded stars in orbit around one another. One star is unidentified, but the companion seems to be a neutron star, an object with the mass of the Sun but shrunk to only a few miles in size, so that its immense gravity causes even atoms to collapse into neutrons under their own weight. Rotating many times a second, this collapsed star emits extremely regular and precise pulses of radio waves (hence the name pulsar) which can be monitored on Earth and used as a built-in clock.

Over the past 4 years astronomers have been able to follow the convolutions of the pulsar in its orbital dance around its invisible companion. Because of the enormous gravitational fields in the system, relativistic effects are extremely prominent. For example, a progressive twisting of the elliptical orbit caused by space-time curvature amounts to a huge 4.2 degrees per year, which should be compared with the celebrated 43 s of arc per century suffered by the planet Mercury.

On page 437 of this issue of *Nature*, three astronomers report measurements on the pulsar taken with the

giant Arecibo radiotelescope in Puerto Rico. The precision of the observations and the quantity of information encoded in the radio pulses is so high that not only can the mass of the pulsar and its unseen companion be deduced, but an internal consistency check can be made on the general theory of relativity. The masses are much as expected, somewhat less than $1\frac{1}{2}$ solar masses, suggesting that both components are neutron stars, but the more exciting result concerns a small, but vitally significant feature of the orbital motion. The astronomers have found that, over the months, the orbital period of the pulsar (which is less than 8 h) is slowly changing. Although the shift only amounts to about one part in 10^9 per year, it is quite distinct and has far-reaching implications.

The systematic drift is readily explained by Einstein's theory. Just as the motion of electric charges produces electromagnetic waves, such as light and radio waves, so the orbital gymnastics of the binary pulsar should produce gravitational waves. In Einstein's theory these are literally ripples of space-time itself, emanating from the central disturbance, transporting energy away from the star system into space. The energy loss is paid for by a decay of the pulsar's orbit, and it is this which the astronomers seem to have spotted. It is similar to deducing the existence of radio waves by observing the rise in one's electricity bill when the transmitter is switched on. Although indirect, the discovery is nevertheless powerful support for the theory of relativity and is bound to attract a lot of scrutiny. Other theories of gravity exist, some of which also predict gravitational radiation, but in many of these the expected effect is considerably larger than that observed. One point which will receive attention is the fact that the relativistic formula that seems to be confirmed is actually only an approximation. The theory of relativity

is mathematically extremely complicated and non-linear, and there is some uncertainty over how accurate the existing approximation really is. There is clearly room for more theoretical work here.

Gravitational waves have long been sought on Earth using metal bars or large crystals as detectors. The principle of these detectors, pioneered by Joseph Weber of the University of Maryland, is to measure a faint ringing sound as the space ripples sweep by. Gravity is so weak that even the strongest bursts of gravitational radiation expected from space will deliver less energy to the bar than would a lighted match held a million miles away. Tremendous advances in technology have been needed to overcome this daunting feebleness, and many physicists, despite some early evidence of success, believe that direct detection of gravity waves here on Earth is still some years away. However, new techniques, and new types of detector such as the laser interferometer, should eventually give birth to the new science of gravitational astronomy, enabling astronomers to look at the binary pulsar and other sources of gravity waves with gravity telescopes.

The last great test of Einstein's general theory of relativity, by Sir Arthur Eddington in 1919, caused a public sensation when it was confirmed that the Sun's gravity bends starlight. The binary pulsar has been the first opportunity for the theory to be tested beyond its first-order corrections to Newtonian gravity, but physicists are treating this latest success with quiet satisfaction. Few seriously doubted the outcome. Nevertheless, it is fitting that the centenary of Einstein's birth should see the confirmation of one of the more intriguing consequences of his work.

P. C. W. Davies is in the Department of Mathematics, King's College, London.

particular advantages of its use can be truly appreciated. For there are no tricky mRNA isolations involved, merely a knowledge of the required peptide sequence. Codon choice seems not to be a problem, as both the human insulin duplexes (incorporating codons chosen from those most frequently found in phage MS2) and the rat proinsulin cDNA have been expressed in *E. coli*. In the future other

peptides and proteins may well be prepared in this way. The DNA sequence need only be synthesised once, Nature's own peptide synthesis mechanism being useable *ad infinitum* (with careful checks for the absence of spontaneous mutations). Moreover, it would be relatively simple to incorporate changes in the amino acid sequences of a protein (by altering the DNA sequence) that might in turn alter its

biological activity.

Whereas synthesis of complete genes still requires a substantial investment of finance and personnel, there are several areas of molecular biology where the availability of short, sequence-specific oligodeoxyribonucleotides has made considerable impact recently. Particularly noteworthy is the use by C. A. Hutchinson *et al.* (*J. biol. Chem.* **253**, 6551; 1978) of a 13-mer

complementary to part of the phage ϕ X174 sequence in all but one nucleotide corresponding to the position of the *am3* mutant. The oligonucleotide was incorporated into a complete, circular, complementary strand, when used as a primer on wild type DNA, with the deliberate G-T mismatch causing an amber mutation in the newly synthesised strand. The use of mismatched oligonucleotide primers could in principle also be used to induce transversions, insertions and deletions, and it is likely that site-specific mutagenesis by this and other techniques will be an area of great future activity.

Synthetic oligodeoxyribonucleotides have also found increasing use in the analysis of DNA structure and function. This topic has been recently reviewed by R. Wu *et al.* (*Prog. Nucleic Acids Res. molec. Biol.* **21**, 101; 1978) and includes applications to DNA sequence analysis, the study of protein-DNA interactions, and the use of oligonucleotides as probes for genes and as tools for molecular cloning of DNA. Indeed the recent dramatic increase in the demand for synthetic DNA has only been partially met by the introduction of more rapid synthetic methodologies. Until commercial enterprise can meet this phenomenal demand some sort of DNA synthesis facility is now an important asset to laboratories involved in molecular biology. $\square$

Moscow revisited—laser fusion 1979

from Ian Spalding

It is eight years since the European Conference on Laser Interaction with Matter (ECLIM) was last held in Moscow. At that time only a handful of visitors from the West contributed to an otherwise well-attended conference; the practical importance for laser fusion of creating highly compressed DT targets (with densities much exceeding those normal in the solid state) was becoming increasingly stressed in the published literature, but the available laser technology was still relatively primitive. As a particular example, the energy then available from a state-of-the-art pulsed CO₂ laser was only 10 J, with a peak power of ~ 10 MW! Of comparable importance, the absorption coefficient for radiation of wavelength λ between $\frac{1}{2}$ and $10 \mu\text{m}$ as a function of incident angle, polarisation and intensity, and as a function of plasma

composition and initial distribution, had not been characterised in systematic experiments—nor were the existing measurements well coordinated with available theoretical (mostly analytic) models of relevant linear and non-linear interactions. Although no fundamental break-through was reported at the twelfth conference in the series held recently*, overall progress in the field has been significant and encouraging.

Advances in laser technology over the past eight years have been particularly striking. 10 kJ, 27 TW, Nd: glass and 10 kJ, 20 TW, CO₂ laser systems have been successfully commissioned in the past year at the Lawrence Livermore and Los Alamos Scientific Laboratories respectively; all of the $1 \mu\text{m}$ and approximately a quarter of the $10 \mu\text{m}$ power has already been symmetrically focused onto gas-filled microballoon 'exploding pusher' targets. DT neutron yield increases with incident Nd laser power to an (unoptimised) 3×10^{10} neutrons at 27 TW; scaling at both wavelengths fits quasi-analytic models. Significant thermonuclear burn will however require the experimental achievement of an isentropic compression of the target's core, so more complex layered microballoon 'ablative burn' targets are now receiving increasing experimental attention. At incident powers $< 10^{12}$ W, the KMS and KALMAR (Lebedev) Nd lasers have demonstrated compressions approaching $\times 35$ (hydrogenic) solid-state densities, using cryogenic targets and α -emission spectroscopy KMS claim that this compression is partly adiabatic. In summary, available 'driver' powers have increased tremendously over the past eight years—for CO₂ lasers by a factor of 10^6 ; ideas advanced for larger systems (regenerative relayed amplifiers, stimulated Raman/Brillouin converters and so on) suggest that 0.1–1 MJ drivers (predicted to be of practical interest) may well be operational within the next decade.

Developments in the understanding of laser-plasma interactions, and of the necessary diagnostic techniques, are equally impressive. Various groups have experimentally investigated the absorption of laser light at wavelengths λ of $\frac{1}{2}$, $\frac{1}{2}$, 1 and $10 \mu\text{m}$, and at intensities I within at least part of the range $I = 10^{10} - 10^{16} \text{ W cm}^{-2}$; there now seems to be universal agreement that strong density-profile modifications are induced by radiation pressure effects when $(I\lambda^2) \sim 10^{15} (\text{W cm}^{-2}) \mu\text{m}^{-2}$, and that resonance absorption significantly increases the total absorption coefficient at the longer wavelengths. Related measurements suggest that much of the absorbed radiant energy is then converted to hot, non-thermal, electrons. Developments in two and

three-dimensional soliton theory, and in analytic modelling of strong turbulence by the Russian school, are equally impressive. The main topic for controversy appears to be the computer optimisation of target designs, where the avoidance of unwanted core pre-heating by the 'hot' electrons and of possible deviations from the desired symmetry of implosion (due to Rayleigh-Taylor instabilities) poses significant modelling problems in the hydrodynamic codes. The US prefer 'thick' (lower aspect ratio) double-shell designs to obviate both of these problems. The increasing tendency of American and Russian theoreticians to 'cross-calibrate' predictions from their codes against published experimental work seems therefore a noteworthy and potentially fruitful development.

It is particularly interesting that Academician E. P. Velikhov took the opportunity of a round-table discussion (including proponents of electron-beam fusion and fission/fusion hybrids) to suggest that the time was ripe for a cooperative international venture in laser fusion, perhaps under the auspices of the IAEA. In making this personal suggestion, he cited the recent IAEA decision to initiate the Intor (joint-Tokamak) study as a bold and historic venture. $\square$

I. J. Spalding is in the Experimental Division A, UKAEA, Culham Laboratory.

Variations on a theme

from Peter Newmark

WITHOUT their analogues, synthetic or natural, insulin experimenters would soon be on the streets. Fortunately the chemist's ingenuity keeps ahead of the biologist's appetite for quantity and variety. For it is through the application of old and new analogues that those attending a recent meeting* saw the eventual solution to most of the mystery still surrounding the actions of insulin.

How exactly, for example, does insulin bind to its cell surface receptor? The combined powers of crystallography and analogue study have outlined the domain on the surface of the insulin which is implicated in binding—a domain which has never been so clear as in the computer-generated molecule presented by P. de Meyts (ICP, Brussels) and R. A. Feldman (National Institutes of Health, Bethesda). (This film was not just a

*ECLIM XII was held in Moscow's Polytechnic Museum on 11–15 December, 1978.

*A European Workshop on Insulin Structure organised at the ICP, Brussels, 13–15 December, 1978 by P. De Meyts and D. Brandenburg.

remarkable travelogue around the surface of insulin but, since its images are generated from molecular X-ray coordinates it heralds a more accurate and far easier method of 'building' models than is now possible). Although the binding domain has been identified, the specific role and importance of individual amino acids within the domain remain uncertain. Certainty may come from new analogues. Perhaps the chemists will succeed in modifying one of the implicated amino acids in a minor way but with drastic consequences for binding. Or perhaps the isolation of a natural insulin analogue from some exotic species will spare the chemist's toils.

It would also help if the receptor for insulin were available in purified form. But that, according to L. Kuehn (Dusseldorf University), will require either a method of affinity chromatography better than any now available, or an alternative, non-classical approach. Matters may be helped by the availability from the laboratory of D. Brandenburg (Deutsches Wollforschungsinstitut, Aachen) of photo-affinity insulin probes for the receptor, which are now being put to use.

Another very recent Aachen contribution is a series of covalent dimer insulins, potentially useful probes of the subunit structure of the receptor. Several varieties of covalent linkage are possible and A. Schuttler described their preparation from insulin molecules with protected end groups.

Early data from the testing of the dimers in various biological systems have contained enough oddities to arouse even the most jaded analogue addict. In the adipocyte lipogenesis assay, for example, the activity of the dimer in which the linkage is between position 1 of the B chain of one molecule and position 29 of the B chain of the other is only very modestly reduced compared with native insulin (R. Jones & P. Sonksen, St Thomas's Hospital Medical School; Schuttler). Also, and against the usual correlative trend, the activity *in vivo* on blood glucose levels of this dimer was much lower than on lipogenesis *in vitro* and, for the activity observed *in vivo*, the blood clearance rate of the analogue was surprisingly high (Jones & Sonksen).

The crystal structure of insulin is of course well established at high resolution but as T. Blundell (Birkbeck College, London) emphasised, crystalline insulin is in hexamer or dimer form which may not provide a good guide to the structure of the biologically active, soluble monomer. Early indications from a variety of sources suggest that insulin does have flexible surface groups whose structure changes considerably when insulin

polymerises contacts. According to Blundell the conformational flexibility seen in the crystalline insulin hexamer is not so evident in its stable-mate, pancreatic polypeptide III, the high resolution structure of which is in the offing.

With an inevitability characteristic of any peptide hormone meeting these days, matters were not complete without the externalisation of internalisation. The most thorough contribution came from J. L. Carpentier (Ecole de Medicine, Geneva). The data he presented showed convincingly that [¹²⁵I] insulin was internalised in lymphocytes and hepatocytes and, with time, accumulated in lysosomes and/or Golgi bodies. The 'point' of internalising insulin (and other hormones) remains enigmatic. There is no evidence for a functional role for insulin within the cell despite the somewhat surprising claim by Carpentier that the internalised hormone is largely undegraded and therefore potentially active. It must be recorded however that data from other less thorough studies was not all consistent

with internalisation.

Finally J. Gliemann (Copenhagen University) showed that insulin mono-iodinated at A14 (tyrosine 14 of the A chain) had twice the affinity of the A19 isomer for the receptor on adipocytes, a factor of such consequence to some experiments that they will bear repeating when the pure A14 isomer is available. Then there is a move to iodinate all analogues and to redetermine their binding parameters by direct instead of indirect means. And there are no end of interesting vertebrates whose insulin has yet to be sequenced—the latest to fall being the casiragua (R. Horuk, Birkbeck College) a rodent relative of the coypu and guinea pig. And still to be answered categorically is the question of whether invertebrates have insulin or some forerunner molecule.

Insulin experimenters, the world is your oyster. Oysters, does your world have insulin? □

Peter Newmark is the Biological Sciences Editor of Nature.

The world's vanishing primates

from Michael Kavanagh

THE International Primatological Society this year held its Congress in Bangalore, India and in one of the satellite symposia* attention was focused on the enormous problems facing primate conservationists in neighbouring Bangladesh. The forests that are the natural home of the country's monkeys and apes are now almost entirely restricted to three areas in the centre and south of the country and they are being destroyed at an alarming rate. None is totally protected and whereas the government estimates Bangladesh's forest area at about 8,600 square miles, this figure is reduced to less than 3,000 square miles when timber monocultures, tea estates, rubber plantations, grasslands, scrub and other man-made vegetation types are excluded. 'Common' langurs (*Presbytis entellus*) seem to be extinct and there are estimated to be only about 35,500 of the most abundant primate, the rhesus monkey (*Macaca mulatta*), left in the forests. A few more survive in the towns, but in contrast to India where wildlife is generally revered, Bangladesh is a country in which most people are ignorant about wild animals and regard them as dangerous or destructive objects to be killed or

persecuted in any way possible. In describing his country's situation, Ali Reza Khan stressed habitat destruction and the lack of education about the natural heritage as the two major threats to primate conservation. Habitat destruction results from the needs of a human population that has reached an average density of 3,000 per square mile of land; and as far as education is concerned, Reza Khan reported that international help is desperately required for such basics as buying the books that Bangladesh cannot afford. He illustrated the Bengali attitude to wildlife with the story of a serow (*Capricornis sumatraensis*), a large, long-legged goat antelope, that wandered over the border from India. It was chased by a mob of thousands of people who attacked it with sticks and stones. Eventually the police intervened and took the animal into protective custody in the local jail until they could hand it over to the Forestry Department.

In describing the plight of Bangladesh's primates and in calling for a moratorium on all exports at least until the primate populations have been thoroughly surveyed (a task in which he is involved), Reza Khan reflected the major concern that dominated the congress. Although the society is an academic body, no academic issue caught the minds of large numbers of

*Satellite Symposium on Primate Population Studies in India, Bangalore, India, 6-7 January, 1979. Part of the VIIth Congress of the International Primatological Society.

delegates in the way that was true of conservation.

Inaugurating the congress, V. Ramalingaswami, President of the Indian National Science Academy, stated that half of the world's 180 non-human primate species were in danger of extinction and he called for a deeper understanding of primate biology in order to facilitate management for survival. He urged biomedical researchers only to use primate subjects where absolutely necessary and pointed out their common interest with conservationists in that both groups desire the preservation of primate populations.

A Conservation Round Table, which was intended to last for a morning, continued by demand of the participants throughout the afternoon with delegates offering summaries of the situation in almost every country that has indigenous primates. In summing up this session in his report to the Business Meeting of the Society, Vice-president J. S. Gartlan struck several themes. First, education about conservation appears to be a major need throughout much of the world. Where efforts have been made, the results have been encouraging. A case in point is Kenya, where the Wildlife Clubs are working successfully in schools. At a higher level, there is a need for the education of field staff (such as game rangers) in the developing countries. This must go hand in hand with the provision of incentives in terms of jobs and security.

Second, the greatest threat to natural primate populations comes from habitat destruction, with trade and hunting for any purpose being of variable seriousness in different areas. He contrasted India where wild animals are seldom molested with West Africa where hunting for protein is a major problem.

Third, the outlook in any area can change rapidly, often due to government action. For example, Guyana still contains large areas of relatively undisturbed forest in its interior, but the government has declared its intention to develop this land for human use including that of providing a site for a new national capital. Gartlan urged continual surveillance of parks and reserves to guard against encroachments or changes in their legal status.

Fourth, management of the ecosystem is essential for a conserved area, and therefore any plans to create a national park or a reserve are worthless without such management.

Gartlan's fifth theme reflected the activist mood of the delegates. He pointed out that most wild primate

The primates of peninsular Malaysia

from David Chivers

THE evergreen rain-forest that covers nearly half the land area of Peninsular Malaysia is rich in primates and other wildlife. There are three species of lesser ape, the siamang (*Hylobates syndactylus*) and lar gibbon (*H. lar*) with the agile gibbon (*H. agilis*) in the north, two species of macaque, the long-tailed macaque (*Macaca fascicularis*) and the pig-tailed macaque (*M. nemestrina*), two species of leaf monkey, the dusky leaf monkey (*Presbytis obscura*) and the banded leaf monkey (*P. melalophos*), (with a third species, the silver leaf monkey (*P. cristata*) in the mangrove swamps of the west coast), and the prosimian slow loris (*Nycticebus coucang*).

Habitat varies with altitude; the lowland Dipterocarp rain-forest has the most varied flora and fauna, and primates abound. The gibbons and leaf monkeys extend up into hill Dipterocarp forest and sub-montane formations, but the smaller gibbons and dusky leaf monkey are more restricted to the lower forests. Pig-tailed macaques show a preference for the higher, more remote forests, while the long-tailed macaques prefer riverine forest and disturbed habitats, in which they scavenge on the by-products of human society.

Forest clearance poses the main threat to Malaysia's wildlife; timber is a valuable resource which has been over-exploited. There is increasing realisation that such benefits are short term, with possible disaster beyond. The maintenance of permanent forest reserve over about 45% of the Peninsula will, it is hoped, contain the increasing problems of flooding and soil erosion. The long-term economic benefit of sustained, light, selective logging, harvesting of other plant products, and cropping of various mammal species in such managed forests, and the benefits of protecting watersheds and organising tourism in undisturbed habitats remain to be established. This needs to be coupled with the more efficient use of agricultural areas.

Until this long-term equilibrium between man and wildlife is established there is the distressing problem of wildlife made homeless by clear-felling. Unless animals can be transferred to other forests where numbers have been reduced by hunting, they are doomed. The Wildlife Department

have shown their interest in tackling this problem, and current research projects will, one hopes, provide guidance.

The Game Department in Malaysia has been reorganised into the Department of Wildlife and National Parks, with the Chief Game Warden, Encik Mohamed Khan bin Momin Khan, KMN, as its Director-General; numerous research officers have been appointed, and a generous allocation of government funds has been made for conservation in the current quinquennium under the Third Malaysia Plan. Apart from coordinating the conservation effort, with encouragement from the Malayan Nature Society, the department is also responsible to the Ministry of Science, Technology and Environment for maintaining national parks and game reserves and enforcing the rigorous laws that protect Malaysia's wildlife. Only *M. fascicularis* is exported at present, at a rate of less than 10,000 a year. More information is needed urgently to assess the effect of this on the wild population, whether the export of any other species can be justified for essential medical research and, if so, in what numbers.

It is against this background that a 3-year programme of collaborative research between Universiti Pertanian Malaysia, Universiti Kebangsaan Malaysia and the University of Cambridge was started in July 1978, assisted with funds from the US Public Health Service, the L. S. B. Leakey Foundation and The Royal Society. The main aims are to study the socio-ecology of the lesser-known species such as the slow loris and the pig-tailed macaque, and to assess the distribution and habitat preferences of each species throughout the peninsula. The intention is to determine precisely the effects of different degrees and types of habitat disturbance on primate populations. Laboratory studies on many aspects of the biology of the indigenous primates will be made using animals displaced by forest clearance. These studies should provide the information essential for their conservation in both natural and disturbed habitats.

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populations are to be found in tropical countries where much development is funded through international agencies. He suggested that modification of plans

at an early stage could often be of benefit to primate conservation and that therefore the International Primatological Society should develop an

early warning system by monitoring international agency intentions. The possibility would then be open for members in areas to be affected to comment constructively to the benefit of conservation requirements.

Finally, he pointed out the failure of many countries to sign the Washington Convention on International Trade in Endangered Species. Austria, Italy and Belgium have not signed, and as a result Europe is a major centre for the smuggling of endangered primates. Similarly, Japan has failed to sign and, according to Gartlan, is a major market for endangered species.

Many delegates volunteered to participate in a network of primatologists who may be called on for a coordinated letter-writing campaign whenever a conservation crisis threatens. The hope is that evidence of worldwide concern will persuade national agencies to modify development plans that threaten primate populations. In my experience, conservation is always a major issue at meetings of the International Primatological Society, but never before has there been such a general sense of urgency. □

Compensation of Doppler-broadening using light shifts

from Peter Knight

A NOVEL technique has been developed by Cohen-Tannoudji and his colleagues at the Ecole Normale Supérieure and Collège de France to produce Doppler-free optical emission with highly anisotropic spectral properties. Normally a cell of excited atoms in random motion will produce an optical field by spontaneous emission whose spectrum is isotropic: no matter which end you look at with a spectrometer, the radiation will be seen to be broadened by Doppler effects to a width typically many times larger than the natural single-atom width. Cohen-Tannoudji has found ways to pump a cell of atoms in random motion with an intense laser field so that the atom can emit (or absorb) radiation without Doppler broadening. The radiation from such pumped atoms is Doppler-free in the forward direction with respect to the laser field with a width governed by the homogenous (natural and collisional) lifetime. In the backward direction the radiation is broad-

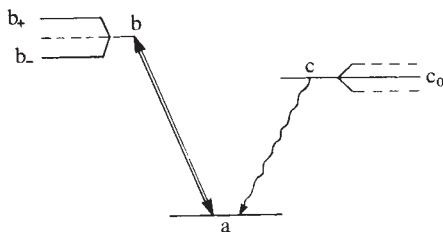


Fig. 1 Energy level scheme for Doppler broadening compensation by light shift perturbations. Levels b and c are Zeeman split by a magnetic field. A circularly polarised laser excites the $ab_{\pm}$ transitions and the linearly-polarised emission from c_0 to a is detected.

ened in excess of the Doppler-width. The process operates by compensating for the Doppler velocity-dependent of transition frequencies by shifting and splitting the atomic levels in a balancing velocity-dependent manner by interaction with a saturating laser field on a connecting laser transition sharing a common lower level (Fig. 1).

Cohen-Tannoudji's first proposal, made at a symposium on frequency standards in 1976, required the use of two saturating laser fields. An optical field detuned by an amount α from a transition frequency of an atom at rest in the laboratory will shift the transition frequency to a new intensity-dependent 'dressed' frequency by a dynamic Stark shift (*Nature* **265**, 683; 1977) which has a dispersion shape. A second laser field detuned an amount α in the opposite direction will produce an equal and opposite Stark shift. The frequency pushing balances the pulling, and for this zero-velocity atom there is no intensity-dependent dynamic Stark shift, or 'light shift'. For an atom with non-zero velocity, then in the atomic rest frame the atom is illuminated by two laser fields with unequal detuning, and the light shifts do not balance out to zero. The laser perturbation of transition frequencies is velocity-dependent, and a careful choice of intensity could counteract the Doppler shift of emission lines.

Together with Hoffbeck and Reynaud, Cohen-Tannoudji (*Opt. Comm.* **27**, 71; 1978) has discussed how the same effect can be produced using one saturating laser field interacting resonantly with two symmetrically detuned atomic transitions separated by a Zeeman splitting (Fig. 1). In Fig. 1, a circularly polarised laser at frequency ω excites the $ab_{\pm}$ and $ac_{\pm}$ transitions which have frequencies $\omega + \delta$ and $\omega - \delta$ where δ is the Zeeman splitting caused by a magnetic field. Each of the transitions light-shifts the level a (and $b_{\pm}$). Again for an atom at rest in the laboratory with $v=0$ these light-shifts cancel, but for $v \neq 0$ the atomic transition frequencies are intensity-dependent and velocity-dependent in a way that can

balance the Doppler shift of emitted lines. For example if the atom sketched in Fig. 1 were weakly excited by a discharge so that radiation were emitted in decaying from another level (way out of resonance with the pump laser on the ab line) c_0 down to a , the emitted light would be Doppler-shifted by a factor $(1-v/c)$ in the forward direction. But if the energy level a were to be light-shifted in a velocity-dependent way as explained, this could shift the ac_0 transition frequency to compensate for the Doppler shift in the forward direction. If this can be arranged for a large enough fraction of the atoms with a spread of velocities, then a significant proportion of the ac_0 transition radiation will be Doppler-free and hence narrowband. Conversely, in the reverse direction the Doppler shift factor is $(1+v/c)$ and the light shift reinforces the Doppler shift, broadening the line to perhaps even double the conventional Doppler width. The special profile of the radiation produced by this 'dressed' three-level system is highly anisotropic. Cohen-Tannoudji *et al.* have determined analytical criteria for Doppler-compensation and discuss the spectra of the emitted light and the way in which the intensity-dependent dressing interactions modify the spectrum of the emitted radiation in the forward direction and produce dynamic-Stark split satellite lines (which they neatly explain in terms of inverse Raman scattering).

Two distinct cases are discussed: partial Doppler compensation for modest laser intensities, where three narrow structures are predicted, and full Doppler compensation for more intense fields where the spectral profile contains a sharp Doppler-free central component and two small Doppler-broadened sidebands. An interesting feature is that the narrowing is entirely due to the energy perturbation of the final state of the transition. The width of the narrow line is governed by parameters such as natural lifetime and collision rates and does not exhibit power-broadening even at high intensities. Other early work on the cancellation of Doppler shifts by a light shift almost linearly dependent on the atomic velocity was reported by Toschek at the 1975 Les Houches meeting (*Frontiers in Laser Spectroscopy* **1**, 329, North Holland, 1977).

Experimental work in progress at ENS using dye laser excitation of ^{20}Ne at 6,163 Å in a magnetic field (to produce the two oppositely detuned Zeeman transitions) and monitoring emission from the third level (c_0 in Fig. 1) has observed the partial Doppler compensation. The emission features in zero magnetic field (a doublet pro-

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duced by the dynamic-Stark splitting of level a by the dye laser field) develops a narrow central feature as the magnetic field is increased, as predicted by Cohen-Tannoudji *et al.* The work has novel implications for Doppler-free

superradiance, the observation of Doppler-free coherent transients, and predicts striking forward-backward asymmetries in spectral profiles of great interest in intracavity ring systems. □

Interferon and double-stranded RNA

from John Morser and Derek Burke

THE *pas de deux* between interferon and double-stranded RNA (dsRNA) continues (see *News and Views* 273, 97; 1978). It has been known for many years that treatment of cells with double-stranded RNA induces interferon. It now seems likely that viruses also induce interferon by virtue of their ability to make double-stranded RNA, as shown by the elegant study of Marcus and Sekellick (*Nature* 266, 815; 1977), although additional factors might also be involved (see Kowal & Youngner *Virology* 90, 90; 1978). When either double-stranded RNAs or viruses are used as inducers, the interferon yield can often be increased by pre-treatment of cells with interferon ('priming'). Priming is specific for interferon and it may often turn a non-inducible system into an interferon-producing system. Some cells, notably mouse L cells, also show a pronounced cytotoxicity when treated with interferon and double-stranded RNA (Stewart *et al. Proc. natn. Acad. Sci. U.S.A.* 69, 1851; 1972). Since such cells also produce interferon, interferon is being synthesised by cells undergoing pronounced cytotoxic damage. Finally, when interferon-treated cells are exposed to double-stranded RNA, translation is blocked, and a series of recent papers (Kerr & Brown *Proc. natn. Acad. Sci. U.S.A.* 75, 256; 1978; Ball & White *Proc. natn. Acad. Sci. U.S.A.* 75, 1167; 1978; Lewis *et al. Eur. J. Biochem.* 86, 497; 1978; Schmidt *et al. FEBS Lett.* 95, 257; 1978; Farrell *et al. Proc. natn. Acad. Sci. U.S.A.* 75, 5893; 1978) shows that at least two separate mechanisms are involved. One involves the activation of a protein kinase that

phosphorylates, and consequently inactivates the initiation factor eIF-2, and the other involves the formation of pppA2'p5'A2'p5'A ('two-five A') which in turn activates a constitutive inactive endonuclease that degrades mRNA (see diagram).

These effects of interferon require the presence of chromosome 21 in human cells, probably to make the interferon receptor on the cell surface. Both pathways that lead to inhibition of protein synthesis require ATP: the pathway resulting in inhibition of peptide chain initiation for the activation of the protein kinase system, and the one resulting in mRNA degradation to synthesise two-five A by the interferon-induced oligo-isoadenylate synthetase. The scheme reveals a paradox: cells in which protein synthesis has been impaired by two separate mechanisms are also the cells in which interferon mRNA is being actively translated. How can a new protein be formed in such cells? Are any of the intermediates involved in the induction of the interferon gene? The enzyme that synthesises two-five A is possibly one candidate, since it complexes sufficiently well with double-stranded RNA for that to be used as a purification method (Hovanessian *et al. Nature* 268, 537; 1977), and seems to be present in small amounts in cells which have not yet been exposed to interferon.

One generalisation can be made, however. Interferon sensitises the cell to double-stranded RNA—so that a series of processes which previously occurred either not at all, or at low levels, is now activated. Perhaps this is the control function of interferon—

to tell the cells to respond to dsRNA—either added as a synthetic dsRNA (poly (rI) poly (rC)) or formed as a by-product of virus multiplication.

It is well known that reticulocyte lysates are exquisitely sensitive to the effect of dsRNA, much more sensitive than are cell-free systems made from normal cells and as sensitive as cell-free systems made from interferon-treated cells. Hunt (*News and Views op. cit.*) has suggested that this is because erythrocyte differentiation depends on exposure to interferon at some stage. Certainly interferon antagonises the effect of colony-stimulating factor, inhibits the growth of haemopoietic colony-forming cells and interferes with the normal differentiation of granulocyte-macrophage precursors (McNeil & Fleming in *The Interferon System: a Current Review to 1978*, Texas Reports on Biology & Medicine (eds Baron & Dianzani) 343; 1978). Interferon also inhibits the expression of erythroid differentiation in Friend leukaemia cells treated with dimethylsulphoxide (Rossi *et al. ibid* page 420).

If interferon is involved in any way in differentiation, is this the reason why the interferon system is an inducible rather than a constitutive system and why it is completely uninducible in mouse teratocarcinoma stem cells (Burke *et al. Cell* 13, 243; 1978)? Perhaps dsRNA is a pleiotropic effector, but only active in interferon-treated cells? We do not yet know the answers to these questions, but perhaps we should look harder for double-stranded RNA elsewhere. □

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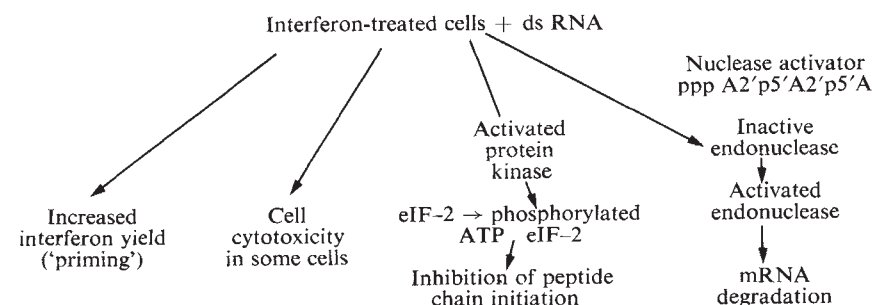
Radioactive waste management

from S. Weisenburger

THIS interdisciplinary meeting*, covering a broad range of topics, from the processing, solidification, and characterisation to the isolation of radioactive wastes arising from the nuclear fuel cycle was devoted especially to scientific research aspects of radioactive waste management. These aspects have often been relegated to the sidelines in international gatherings chiefly concerned with systems management.

A general review on the management of nuclear waste by Rustum Roy

*An International Symposium on 'Science underlying waste management' was held in Boston on 28 November–1 December, 1978 and sponsored by the Materials Research Society. The proceedings will be available in Spring, 1979.



(Pennsylvania State University) ranged from the selection of suitable matrix materials for different types of nuclear wastes to geological considerations relevant to nuclear waste storage.

Several schemes of radioactive waste solidification are currently under consideration. Most papers dealt with matrix materials for high-level radioactive waste (HLW). The technologically most advanced solidification process for HLW is vitrification, which is already in use (for example the French AVM process). However, there are still very intensive research and development efforts aimed at putting the waste into solid forms other than glass, thus providing even better barriers.

Processes based on multibarrier encapsulation concepts were also presented. An alternative approach is to incorporate the radionuclides into minerals (or a synthetic host simulating a mineral) known to have been stable for millions of years in the past. The fixation of HLW in cermet form with an iron-nickel base was also considered. Multibarrier encapsulation relies on up to three barriers to isolate radionuclides from the environment: a solid waste inner core, an impervious coating and a metal matrix. Two candidate materials for the inner core have been suggested: sintered ceramic and glass marbles. If it were desirable to produce a waste form with enhanced inertness (compared to monolithic glass) but with minimum technological complexity, then glass marbles encapsulated in a vacuum-cast lead alloy was recommended for further development.

An example of incorporating HLW into minerals is the SYNROC-process which has been developed on a laboratory scale. SYNROC is a synthetic rock composed of phases such as hollandite, cubic zirconia, perovskite and celsian and its bulk composition is characterised by a high content of TiO_2 (35%) and a low content of SiO_2 (12%). Calcined HLW can be added to SYNROC-melts and the mineral assemblage can be formed by direct crystallisation from a melt cooled below 1300°C , or by hot pressing under subsolidus conditions at $1100\text{--}1250^\circ\text{C}$. The product is considered to be highly resistant to alteration and leaching in any geological environment. The SYNROC process is far from ready to be put into practice in an engineering sense and some important aspects are not entirely clear.

Many papers dealt with the characteristics of nuclear waste in forms including glass, glass-ceramic,

ceramic, minerals, cement, and concrete. Besides microstructural characterisation, the measurements of leach rates and leach mechanism of solid radioactive waste forms were of major concern. Detailed knowledge about leaching is required to establish which are the hazardous radionuclides if and when the waste comes into contact with a leaching medium. The specific radionuclides of concern are relatively few (^{137}Cs , ^{90}Sr , rare earths, actinides). It was shown that the leaching process can be highly complex and that the chemistry of nuclear waste glass reactions must take into account a multitude of diffusing species, as well as changes in the leach interface, and that the solvent chemistry may not remain constant throughout a test run. Expressions were given which fit the leach data and which were based on diffusion models in combination with other influences (time-dependent surface concentration of mobile species, dissolution and precipitation of mobile species, dissolution of matrix material). A relatively new method was described which allows measurements of compositional profiles in the leached waste material using ion beam spectrochemical analysis.

The main subject of the presentations dealing with radioactive waste in geological settings was the detailed analysis of the interaction of waste glass with the geological environment. The most credible scenario for rapid mobilisation of the radionuclides contained in the waste is dissolution due to mobility of indigenous water in the host rock or intrusion of ground water in the repository. The combination of radioactive decay heat (in the first few hundred years) and pressure would then create a 'hydrothermal' environment (up to 400°C , 300 bar) that may accelerate corrosion and breaching of canisters. Results of experimental work show that the hydrothermally reacted glass forms complex products. Under the applied experimental conditions the glass tends to convert to a substantial extent into crystalline phases (50%), the major component being $\text{NaFeSi}_2\text{O}_6$. Bulk X-ray fluorescence analysis of the surface of the reacted glass had shown that Cs, Rb, and Mo were significantly leached into the aqueous phase. Methods were proposed to protect reactive waste from hydrothermal conditions or to produce waste forms which can withstand such conditions (multibarrier waste isolation shows significant promise).

Thirteen papers dealt with radionuclide transport phenomena in geomedias. There is still a paucity of quantitative estimates of the various geological materials' ability to retard nuclide movement, but progress does

appear to have been made in establishing a data bank for sorption and migration of radionuclides in different geological media. These data are urgently needed as part of the input for modelling and safety assessment of geological waste repositories. A number of thermodynamic as well as kinetic parameters influence sorption and migration data and it is now being realised that the numerous experimental designs used for their measurement should be replaced by standard techniques. Any prediction of the long-term sorption and migration behaviour of the radionuclides, must take into account the effects of specific variables such as permeability, porosity, cation exchange capacity, particle size and surface area.

Modelling and safety assessment is mainly concerned with detailed analyses of the full spectrum of phenomena which alone or in consort, could conceivably lead to significant leaching. Results were presented from a pilot computer program based on a model for a geological system containing a waste repository.

To summarise, the symposium showed that extensive scientific research is being done in the field of nuclear waste management. However, it is also clear that much more research and development is required before the behaviour of radioactive waste is fully understood. $\square$



A hundred years ago

WE learn from the *Journal de St. Petersburg* that the epidemic in Astrakhan was discussed before the Russian Medical Society at a gathering where 800 were present. It seems that the people call it the plague, though it is not officially so known. M. Botkine mentioned that at the time of the last plague at Moscow in 1770, the question was discussed whether it was the true plague or a marked form of typhus, and he added that the diagnosis of the various forms of typhoid infection in Russia is very difficult. He believes that the spots on the body and the quickness with which death follows indicate that the present epidemic of Vetlianka is not a European malady. Dr. Nicolaiew, describing the symptomatology of the plague, said that its action is both physical and moral, and that to impose quarantine often helps rather than retards the spread of the disease by the fear it awakens.

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articles

Measurements of general relativistic effects in the binary pulsar PSR1913+16

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Measurements of second- and third-order relativistic effects in the orbit of binary pulsar PSR1913+16 have yielded self-consistent estimates of the masses of the pulsar and its companion, quantitative confirmation of the existence of gravitational radiation at the level predicted by general relativity, and detection of geodetic precession of the pulsar spin axis.

THE earliest observations of binary pulsar PSR1913+16 showed that, because its orbit involved large velocities ($v/c \approx 10^{-3}$), a high eccentricity ($e \approx 0.617$), and relatively strong gravitational fields ($GM/c^2 r \approx 10^{-6}$), several special and general relativistic effects should eventually be observable¹. The advance of periastron (at the rate $\dot{\omega} \approx 4.2 \text{ deg yr}^{-1}$) was the first of these effects to be measured², and the rate of advance has now been determined to better than 0.1% accuracy^{3,4}. We report here the detection of four more effects of relativistic origin, including quantitative measurements of three of them. Together with the much larger effects already measured, the new parameters over-determine the system and provide: (1) the first determination of the mass of a radio frequency pulsar; (2) constraints on the nature of the companion star, and a measurement of its mass; (3) determination of the angle of inclination between the plane of the orbit and the plane of the sky; (4) quantitative confirmation of the existence of gravitational radiation at the level predicted by general relativity; and (5) qualitative observation of geodetic precession of the pulsar spin axis. The data are consistent with the general theory of relativity and provide some strong constraints on any alternative theory of gravitation.

Pulse arrival-time data

Most of the new information comes as the result of pulse arrival-time measurements, which now comprise approximately 1,000 observations spanning 4.1 yr. The data were obtained at frequencies near 430 and 1,410 MHz, using the 305-m radio telescope at Arecibo, Puerto Rico. The normal observing procedure involves adding together approximately 5,000 pulses, using a pre-computed ephemeris to define the expected pulsation period. The resulting mean pulse profile is then fitted by the method of least squares to a long-term average 'standard profile', to obtain the precise pulse arrival time. Because of improvements in equipment and techniques between 1974 and 1978, our timing accuracy has gradually improved³⁻⁵ from

~1 ms to ~50 μs . Data have been acquired at intervals not exceeding 7 months, and in spite of the short period of the pulsar ($P = 0.059 \text{ s}$), there has been no problem in keeping track of the number of elapsed pulse periods.

Our analysis of the timing data follows the formulation of Epstein⁶, and proceeds by the following steps. First, the pulse arrival times are corrected from the location of the observatory to the barycentre of the Solar System, including a relativistic clock correction to account for annual changes in gravitational potential at the Earth. A correction is then made for the dispersive delay in the interstellar medium, using the frequency of observation as Doppler-shifted by the Earth's motion. Finally, the proper time t_p in the pulsar's reference frame is obtained by correcting for (1) the projection onto the line of

Table 1 Parameters derived from timing data

Right ascension (1950.0)	$\alpha = 19 \text{ h } 13 \text{ min } 12.474 \text{ s} \pm 0.004 \text{ s}$
Declination (1950.0)	$\delta = 16^\circ 01' 08''.02 \pm 0''.06$
Period	$P = 0.059029995269 \pm 2 \text{ s}$
Derivative of period	$\dot{P} = (8.64 \pm 0.02) \times 10^{-18} \text{ s s}^{-1}$
Projected semimajor axis	$a_1 \sin i = 2.3424 \pm 0.0007 \text{ light s}$
Orbital eccentricity	$e = 0.617155 \pm 0.000007$
Binary orbit period	$P_b = 27906.98172 \pm 0.00005 \text{ s}$
Longitude of periastron	$\omega_0 = 178.864 \pm 0.002^\circ$
Time of periastron passage	$T_0 = \text{JD } 2442321.433206 \pm 0.000001$
Rate of advance of periastron	$\dot{\omega} = 4.226 \pm 0.002 \text{ deg yr}^{-1}$
Transverse Doppler and gravitational redshift	$\gamma = 0.0047 \pm 0.0007 \text{ s}$
Sine of inclination angle	$\sin i = 0.81 \pm 0.16$
Derivative of orbit period	$\dot{P}_b = (-3.2 \pm 0.6) \times 10^{-12} \text{ s s}^{-1}$

Quoted uncertainties are twice the formal standard errors from the least-squares fit.

sight of the pulsar's orbital position, (2) the integrated effects of gravitational redshift and transverse Doppler shift in the highly eccentric pulsar orbit, and (3) the gravitational propagation delay. These three corrections have magnitudes of order (v/c) , $(v/c)^2$ and $(v/c)^3$ times the orbital period, respectively. On the basis of initial guesses for the various parameters that enter the timing equation (see Table 1), the pulsar phase at the time of observation can be predicted from the relationship

$$\phi = \phi_0 + t_p/P - \dot{P}t_p^2/(2P^2) \quad (1)$$

This phase should be very close to an integer; the difference, or phase residual in periods, is used in a least-squares solution for improved values of the model parameters.

As indicated in Table 1, our timing model contains 13 parameters of physical interest, all of which must be determined

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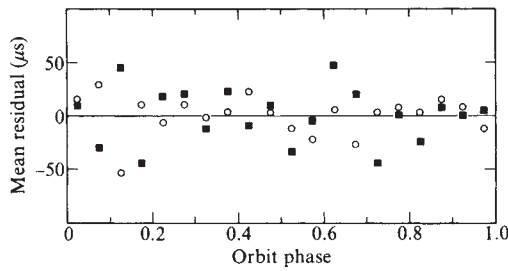


Fig. 1 Differences between observed and computed pulse arrival times for PSR1913+16, after averaging the data into 20 equal intervals of orbit phase for each of two observing frequencies: ■, 430 MHz; ○, 1,410 MHz. Parameter values listed in Table 1 were assumed for the computations, together with a dispersion measure of $171.64 \text{ cm}^{-3} \text{ pc}$.

from the timing data. The parameters include the celestial coordinates of the system α and δ ; the period and period derivative of the pulsar 'clock', P and $\dot{P}$; five Keplerian orbit parameters; and four parameters which measure effects of special and general relativistic origin. In addition to the advance of periastron, the relativistic terms include γ , which measures the variable part of the time delays contributed by transverse Doppler shift and gravitational redshift; $\sin i$, the sine of the angle of inclination between the orbit and the plane of the sky, which is measurable because of its influence on both the gravitational propagation delay and the post-newtonian corrections to the elliptical orbit; and $\dot{P}_b$, the rate of change of orbital period. Further details concerning the timing model and methods of treating the data may be found elsewhere^{3,6-8}.

Parameter values based on data acquired through October 1978 are listed in Table 1. The weighted root mean square deviation of the 837 arrival-time measurements about the fitting function is $80 \mu\text{s}$. In an attempt to search for possible low-level systematic departures of the data from the model, the residuals were averaged in 20 equally spaced segments of orbit phase. The resulting phase-averaged residuals are plotted in Fig. 1, with 430 MHz data and 1,410 MHz data shown separately. The points seem to be scattered at random about the zero line, with no phase dependent errors exceeding $\sim 20 \mu\text{s}$. We conclude that the timing model being used provides an exact fit to the data, within our measurement uncertainties, and therefore that no significant effects have been omitted from the analysis.

Except for $\dot{\omega}$, the relativistic parameters have not previously been measurable to useful accuracies. As shown in Table 1, all of them have now been determined to within $\leq 20\%$ accuracy. The

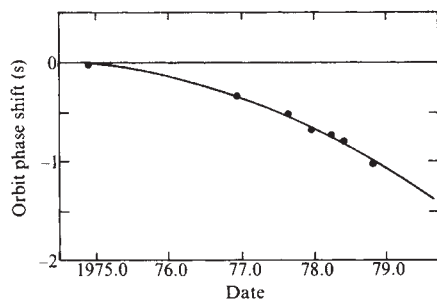


Fig. 2 Curves to delimit possible masses of the pulsar and its companion, based on the observed values of γ , $\sin i$ and $\dot{P}_b$. On these criteria, the most probable masses lie in the shaded region. The sloping straight line is the locus of all mass pairs for which the general relativistic contribution to $\dot{\omega}$ is the observed value, $4.266 \text{ deg yr}^{-1}$. The emphasised portion of this line is consistent with the measured values of γ and $\sin i$ and very close to the measured value of $\dot{P}_b$; it probably represents the best available estimates of the masses.

most uncertain of the four quantities is $\sin i$, because the terms in the timing equation on which it depends have the smallest amplitude^{6,7}, of order $P_b(v/c)^3 \approx 30 \mu\text{s}$. Because this amplitude is somewhat smaller than the typical individual measurement uncertainties, we performed an F-test for the significance of including $\sin i$ in the fit. The test gives a positive result at the 95% confidence level, and thus we believe that the value quoted for $\sin i$ is valid.

Together with the well determined Keplerian parameters, the values of γ , $\sin i$ and $\dot{P}_b$ provide strong constraints on the masses of the pulsar and its companion (m_p and m_c , respectively). These constraints can be illustrated conveniently in a diagram of pulsar mass against companion mass, as shown in Fig. 2. In constructing this figure we have used the relationships

$$\gamma = 0.002951 \left(\frac{m_c}{M_\odot} \right) \left(\frac{m_p + 2m_c}{M_\odot} \right) \left(\frac{m_p + m_c}{M_\odot} \right)^{-4/3} \text{ s} \quad (2)$$

$$\sin i = 0.5083 \left(\frac{m_c}{M_\odot} \right)^{-1} \left(\frac{m_p + m_c}{M_\odot} \right)^{2/3} \quad (3)$$

$$\dot{P}_b = -1.70 \times 10^{-12} \frac{m_p m_c}{M_\odot^2} \left(\frac{m_p + m_c}{M_\odot} \right)^{-1/3} \text{ s s}^{-1} \quad (4)$$

$$\dot{\omega}_{\text{GR}} = 2.11 \left(\frac{m_p + m_c}{M_\odot} \right)^{2/3} \text{ deg yr}^{-1} \quad (5)$$

which follow directly from equations summarised in ref. 3 and the parameters given in Table 1. Curves have been plotted in Fig. 2 bracketing the range of values of γ , $\sin i$ and $\dot{P}_b$ consistent with the data; they suggest a combination of pulsar mass and companion mass somewhere within the shaded region, that is $m_p \approx m_c \approx 1.6 M_\odot$. According to equation (5), one would then expect to observe $\dot{\omega} = 4.58 \text{ deg yr}^{-1}$. Because the observed value of $\dot{\omega}$ is well determined at $\sim 10\%$ less than this value, it seems that a much more probable solution for the masses lies somewhere along the emphasised portion of the sloping straight line, such that $m_p = 1.39 \pm 0.15 M_\odot$, $m_c = 1.44 \pm 0.15 M_\odot$, and that the observed rate of periastron advance is entirely caused by the general relativistic effect. In this case, the observed magnitude of $\dot{P}_b$ agrees with the value expected from gravitational radiation damping to within a factor 1.3 ± 0.3 . There is no compelling evidence for significant tidal or rotationally-induced contributions to $\dot{\omega}$.

Undoubtedly the most important new result of this work is the measurement of $\dot{P}_b$ with the sign and magnitude expected on the basis of gravitational radiation within general relativity. The validity of the measurement is clearly shown in Fig. 3, which shows the accumulating orbit phase error caused by assuming $\dot{P}_b$ fixed at its 1974.9 value, that is $\dot{P}_b = 0$. The measured points (which correspond to separate determinations of the time of periastron passage, T_0 , for each of seven major observing sessions) are not well fit by a straight line. However, they fall very close to the plotted parabola, which represents the general relativistic prediction for $m_p = m_c = 1.41 M_\odot$ according to equation (4). The data thus provide a striking confirmation of a longstanding prediction of the general theory of relativity, and an indirect proof of the existence of gravitational waves carrying energy away from the orbiting system.

Other possible contributions to $\dot{P}_b$ seem to be implausible, *ad hoc*, or of negligible magnitude. For example, differential galactic rotation and mass loss from the system contribute at most about $\sim 1\%$ (refs 9, 16, 21) of the observed value of $\dot{P}_b$. If the (as yet unidentified) companion object is a neutron star or a black hole, then tidal dissipation is also of no significance. Tidal effects could be important in a white dwarf companion, but the only white dwarfs consistent with $\dot{\omega} = 4.266 \text{ deg yr}^{-1}$ have either rapid rotation⁹ or masses close to the Chandrasekhar limit. Such stars would produce either positive or negligible contributions^{9,22,23} to $\dot{P}_b$. We conclude that the only straightforward interpretation of the observed orbital decay is in terms of gravitational radiation damping.

Gravitational radiation

It has already been pointed out^{10,11} that most theories of gravity other than general relativity predict dipole gravitational radiation from an orbiting system like PSR1913+16. In these theories, the predicted dipole radiation is considerably larger than the quadrupole radiation in general relativity, and in some theories it even carries energy of the opposite sign¹¹. Unless the two orbiting stars are within a few per cent of being identical in their ratios of gravitational binding energy to inertial mass (in which case the dipole radiation term vanishes) it is difficult to reconcile our measurement of $\dot{P}_b$ with any such theory. The data

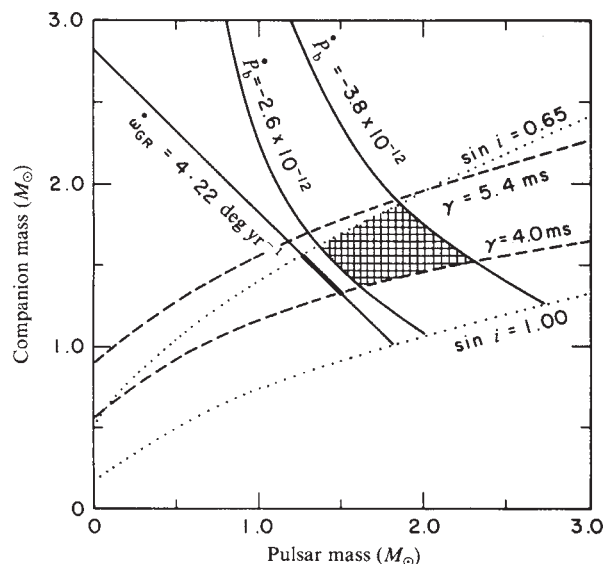


Fig. 3 The points represent measured orbital phase errors caused by assuming a fixed value of P_b . Uncertainties associated with each point are comparable with or smaller than the point itself. The plotted curve corresponds to the orbital period derivative predicted by general relativity if $m_p = m_c = 1.41 M_\odot$.

are also inconsistent with the suggestion that gravitational waves may be composed of half-retarded, half-advanced potentials, and would therefore carry no energy at all¹².

An argument has been put forth^{13,14} that a formula for energy loss due to gravitational radiation has not yet been derived consistently within general relativity, and that the quadrupole formula used^{15,16} to derive equation (4) may be inaccurate because it ignores nonlinearities caused by radiation-reaction forces. As shown in Fig. 2, our data suggest that any such inaccuracy is not very large. The uncertainty associated with $\dot{P}_b$ will decrease rapidly with continued measurements over the next few years, so it will soon become evident if there is any significance to the small apparent discrepancy.

The new mass determinations are consistent with current ideas about neutron star structure and formation and with the hypothesis, based on evolutionary grounds, that the binary system consists of two neutron stars^{9,17,18}. A main sequence helium star¹⁹ can now be ruled out as a companion, because it would require $\dot{P}_b \approx -0.5 \times 10^{-12}$, about one-sixth of the observed value.

General relativity also predicts^{15,20,24} that in this system geodetic spin-orbit coupling should cause the pulsar spin axis to precess at a rate $\dot{\Omega}_p \approx \frac{7}{24} \dot{\omega}_{GR} \approx 1 \text{ deg yr}^{-1}$. According to currently viable pulsar theories, a double-peaked pulse shape such as that observed for PSR1913+16 is probably caused by the rotation through our line of sight of a hollow cone of beamed emission. Thus, as the spin axis precesses, we should expect to observe different cuts through the radiation beam, probably accompanied by changes in pulse width and/or shape.

Such an effect has now been observed, as shown in Fig. 4. Here we plot the average pulse profiles observed in July 1977,

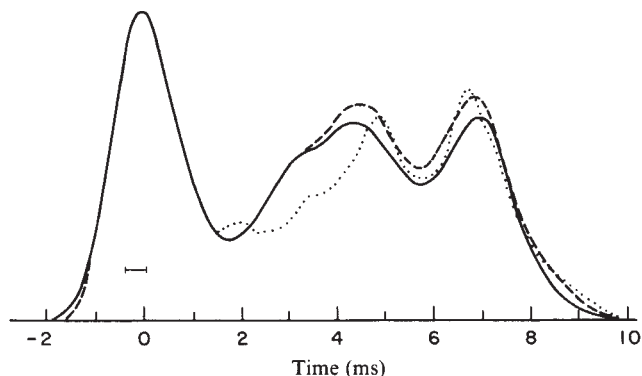


Fig. 4 Pulse profiles observed at 430 MHz in July 1977 (dotted line) and June (dashed line) and October (solid line) 1978. The central component has been gradually moving to the left and becoming broader, while the third component has shifted to the right. All profiles have been smoothed to the resolution indicated by the horizontal bar, 400 μs .

June 1978 and October 1978, with the 1978 data smoothed so as to have the same time resolution (400 μs) as the earlier profile. The three profiles have been aligned at the leading edge and normalised so that the first component has identical height in all three cases. With this convention, the leading component does not seem to have changed significantly, but the central component has broadened substantially and moved towards the pulse centre. At the same time the separation between outermost peaks has progressively increased, and the third component has also broadened somewhat. The extreme trailing edge of the pulse seems to fall more rapidly in the October 1978 profile than in the earlier ones. However, this trailing edge is considerably wider than that observed⁴ at 1,400 MHz, probably because of interstellar scattering. Consequently, this part of the pulse shape change may result from a change in the scattering time constant of the interstellar medium.

Changes of pulse shape towards a broader and more symmetrical profile suggest that precession is moving the observer's line of sight closer to the centre of the radiation cone. A geometry that accounts qualitatively for the observed changes is shown schematically in Fig. 5. More data will be required over a longer time interval, preferably including polarisation

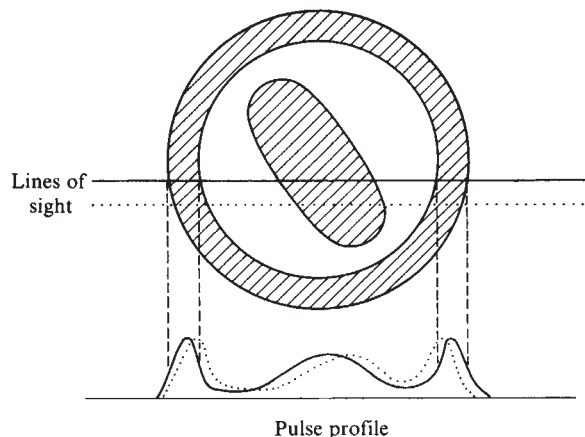


Fig. 5 A possible geometry to account for the pulse shape changes shown in Fig. 4. Shaded regions represent active portions of a hollow cone of pulsar emission; horizontal lines represent loci of the line of sight through the beam as the pulsar spins. Hypothetical pulse profiles are sketched below. As the spin axis precesses, moving a different portion of the beam into the line of sight, the profile is expected to change. October 1978 (solid line); July 1977 (dotted line).

information, before any quantitative description of the observed changes of angle can be made.

Our measurement of the rate of change of orbital period is an entirely new test of general relativity, and at present the only one that depends on the theory's structure beyond the first post-newtonian approximation. The only straightforward interpretation of the data is that gravitational waves exist and carry energy away from an orbiting system at the rate predicted by general relativity. The conclusions that $m_p \approx m_c \approx 1.4 M_\odot$ and $\dot{\omega} \approx \dot{\omega}_{GR}$ give strong support to the interpretation of the

PSR1913+16 system as a pair of neutron stars, and provide an important test of the theory of late stages of stellar evolution. Finally, the observed precession of the pulsar spin axis provides a unique opportunity to obtain information on the shape of the pulsar radiation beam in two dimensions.

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Vulcanian eruption mechanisms

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Application of a newly developed theory to volcanological observations of explosive eruptions shows that previous estimates of pre-explosion gas pressures may be overestimated by an order of magnitude.

THE andesite stratovolcano Ngauruhoe, in North Island New Zealand, erupted explosively during the afternoon of 19 February 1975. The eruption was closely observed and filmed¹. There were two main phases. A 1.5-h gas-streaming phase² produced an 11–13 km high eruption column. This phase was followed by a series of discrete cannon-like explosions at 15 to 50 min intervals over a period of 5 h (ref. 2). The latter type of activity is common on many andesite and basaltic andesite volcanoes, for example Asama³ and Arenal⁴, and is referred to as vulcanian type activity^{5,6}.

Vulcanian explosions have been studied from several recent eruptions^{7,8} and display common characteristics: the rock mass ejected per explosion is usually 10^2 to 10^6 tonnes (refs 3, 6) and often contains a high proportion ($\geq 50\%$) of nonjuvenile material; during active periods, intervals between explosions vary from <1 min (Anak Krakatoa⁹) to about 1 day (Asama³, Sakurazima⁹); pyroclastic avalanches (*nuées ardentes*) are often produced^{8,10,11}.

Nairn and Self² recently described the February 1975 eruption of Ngauruhoe and interpreted the generation of cannon-like explosions in the light of concepts established in the vol-

canological literature. Here we use newly developed theory to re-analyse the largest of the cannon-like explosions (at 18.10 h on 19 February) and discuss an alternative model of vulcanian explosions. The model is constrained by paucity of data, particularly on lava tensile strengths and weight percentages of fine ejecta and released gases, but does point out the type of data required for improved analysis of vulcanian eruptions. McBirney¹² recently reviewed controls on explosive andesitic eruptions and criticised the use of the Bernoulli equation to derive pre-explosion pressures. However, use of the 'gun-barrel' equation, recommended by McBirney, is also open to criticism. This article revises some earlier computations² of pre-explosion pressures at Ngauruhoe and demonstrates that gas pressures required to generate vulcanian explosions are probably lower than previously thought.

Direct observations of discrete vulcanian explosions

The best documented discrete explosion at 18.10 h consisted of the expulsion of a cloud of gas containing fragments with a wide range of sizes. The initial velocity was supersonic, resulting in a shock wave². The position and extent of the cloud was recorded on a series of photographs timed with sufficient accuracy for estimates to be made of the average velocity between each pair of photographs. Figure 1 shows the resulting variation of velocity with height; an estimate of errors has been included.

It is known that the velocity of a cloud of material ejected into the atmosphere at high speed must decrease approximately exponentially with height¹³ after passing through a maximum value, thus one estimate of the initial velocity can be obtained by fitting a straight line to the data shown in Fig. 1: the value indicated is $\sim 400 \text{ m s}^{-1}$. A second estimate comes from consideration of the dynamics of the shock wave propagation: to produce the apparent shock velocity of at least 600 m s^{-1} the velocity of the ejected slug of debris would have to be not less than 340 m s^{-1} (ref. 2) calculated for a one-dimensional case. The true maximum velocity of the ejecta probably lies between these values.

Theory of vulcanian explosions

Discrete, intermittent explosions, with the exception of phreatomagmatic explosions, must be the result of the sudden release of pressure in a gas by the failure of some form of retaining medium. In the vulcanian case the gas may be magmatic or meteoric. It seems improbable that a layer of unconsolidated clasts at the top of the conduit can substantially retain the build-up of an appreciable gas pressure. We assume, therefore, that magma is always involved in such events. New magma rises in a conduit after a previous explosion and cools at its upper surface, forming a 'cap'.

The compressional strength of dolerite is known to remain nearly constant¹⁴ with temperature up to 950°C ; in view of the lack of evidence to the contrary, we assume that tensile strengths of rocks, commonly quoted to be of the order of 10% of compressional strengths, also remain constant up to this temperature. Tensile strengths (at low pressures) of 100–200 bar are deduced for rocks of andesitic type from the limited data available^{14,15}. To obtain an estimate of the thickness of crust sufficiently cool to provide a strength of this order, standard treatments on heat flow can be used¹⁶. Cooling from magmatic temperature (say $1,000^\circ\text{C}$) during the 50 min interval before the 18.10 h explosion could have produced a layer, cooler than 950°C , which was up to 15 cm thick, the distance a thermal wave can travel in rock in this time. Clearly, cool fragments of rock debris smaller than 30 cm in diameter would be efficient heat sinks on this time scale. Indeed, the addition of 10% by weight of such debris, derived from the conduit walls or the fall-back in the vent area from earlier explosions, would cool a layer of any desired thickness to 950°C .

The pressure in a mixture of degassing magma and heated groundwater prior to an explosion would be equal to the tensile strength at depth H below the cap by an amount $\sigma_r g H$ where σ_r is the magma density and g the acceleration due to gravity. At a depth of 500 m in partially degassed magma of bulk density $1,000 \text{ kg m}^{-3}$ the added pressure would be about 50 bar.

Many attempts have been made to relate the ejection velocity of fragments from volcanic explosions, particularly those of vulcanian type, to the initial pressure of compressed gas driving the explosion^{6,8,12,17,18}. All of these treatments are essentially based on a modified form of the Bernoulli equation. We are no longer satisfied that this equation adequately represents the conditions in any actual volcanic explosion.

The simplest treatment of an explosion driven by expanding gas is one in which it is assumed that complete decompression to atmospheric pressure occurs, and that all of the internal energy released by the expansion is used to accelerate the explosion products, both released gas and clasts, to the same velocity. The energy equation¹⁹ is then:

$$-\int \frac{dP}{\rho} = \int g dh + \int u du \quad (1)$$

in which ρ is the bulk density of the eruption products, P is the pressure, g is the acceleration due to gravity, h is the vertical coordinate and u the velocity. ρ must be defined in terms of n , the weight fraction of released gas in the exploding mixture, σ ,

the gas density, and σ_r , the clast density:

$$\frac{1}{\rho} = \frac{n}{\sigma} + \frac{1-n}{\sigma_r} \quad (2)$$

σ is in turn dictated by the temperature, pressure and composition of the gas; in the present circumstances it is adequate to assume that the perfect gas law applies. It is necessary, before integrating equation (1), to decide whether the gas expansion will be more nearly isothermal or adiabatic; the deciding factor is the efficiency with which heat can be transferred from clasts to gas and depends on the clast size distribution²⁰. In the case of strombolian explosions, in which the time scale is very short, an adiabatic approximation is valid¹³. For vulcanian explosions, the best solution probably lies between the isothermal and adiabatic cases. The results of integrating (1) using (2) and an initial temperature T_i are:

$$nRT_i \ln(P_i/P_t) + \frac{(1-n)}{\sigma_r}(P_i - P_t) = g\Delta h + \frac{1}{2}u_t^2 \quad (3)$$

in the isothermal case and, in the adiabatic case:

$$nRT_i \frac{\gamma}{(\gamma-1)} \left[1 - \left(\frac{P_t}{P_i} \right)^{(\gamma-1)/\gamma} \right] + \frac{(1-n)}{\sigma_r}(P_i - P_t) = g\Delta h + \frac{1}{2}u_t^2 \quad (4)$$

where P_i is the initial pressure driving the explosion, P_t is the final pressure, assumed equal to atmospheric, Δh is the vertical

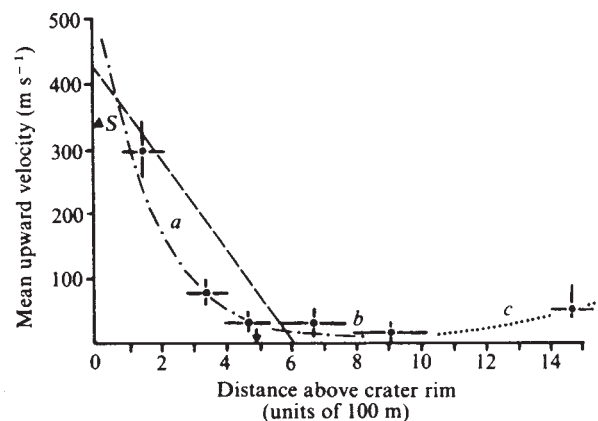


Fig. 1 Deceleration of 18.10 h plume from analysis of still photographs. Errors shown as bars. Straight line fitted between maximum and minimum values; curved line approximates exponential deceleration. S is speed of sound in air for reference. The steep portion of curve (a) represents deceleration in gas thrust phase; the flat portion (b) represents slow, stable velocity condition while mixing with air and column collapse takes place; (c) is a slight increase in velocity at beginning of convective thrust phase. The downwards pointing arrow indicates onset of column collapse.

distance over which the gas decompression occurs, γ is the ratio of the specific heats of the gas, R is the gas constant and u_t is the final velocity. If numerical values are inserted, it is generally found that the term $g\Delta h$ can be neglected for explosions in which the expansion of gas occurs over a vertical distance of less than a few hundred metres.

The modified version of the Bernoulli equation is usually written:

$$P_i - P_t = \frac{1}{2}\sigma_r u_t^2 \quad (5)$$

in terms of the variables already defined. There has been some discussion¹² as to what density should appear in this equation: σ_r (as written here) or ρ . Comparison of equations (5) and (3) reveals the essential problem with the modified Bernoulli equation: it only approximates the correct expression if n is

sufficiently small (and if Δh is also small). The values of n encountered in real volcanic explosions (say 0.1 to 30 weight per cent if plinian, vulcanian and strombolian events are included) are always too large for this approximation to be true.

We have argued above that the build-up of pressure beneath a restraining cap may be the source of vulcanian explosions. In such a case the mixing of gas and clasts from the disrupted cap and the surrounding rocks may not be great in the early part of the motion; a physical model based on the motion of a solid block of coherent (or shattered) rock overlying a gas body of

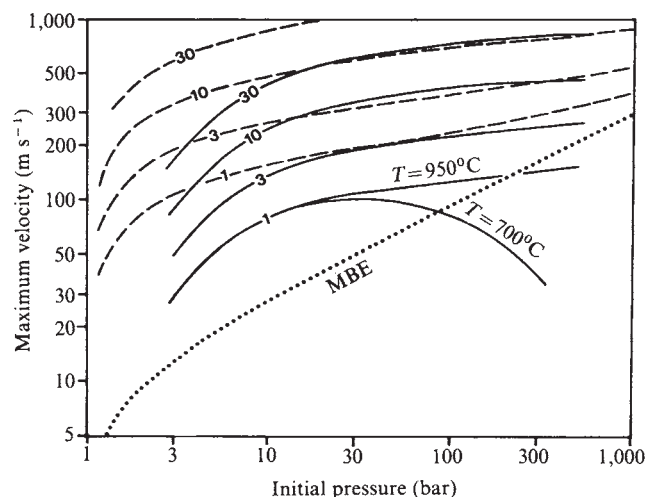


Fig. 2 Maximum velocity of explosion products as a function of initial pressure driving the gas expansion for various types of volcanic explosions. The dotted line MBE is the modified Bernoulli equation (equation (5)). The dashed lines are calculations using equation (3) and are labelled by the wt % gas (taken to be steam) used in the calculations. For these curves perfect thermal contact between gas and clasts is assumed. The solid curves, also labelled by wt % gas in the exploding mixture, represent solutions to equation (7); the gas cools adiabatically during its expansion from an initial temperature of 1,220 K; in the case of the curve for 1% gas, the effect of reducing the initial temperature by 250 degrees is shown. The dashed and continuous curve for each gas content bracket the upper and lower limits of velocity for a given driving pressure in vulcanian explosions. When the ejecta are predominantly less than 10 mm in diameter, velocity values correspond more closely to the dashed curves.

similar cross sectional area may be more applicable. If the vertical extent of the cap is L and its density is σ_r , and if the gas body beneath the cap has vertical extent X , initial temperature T_i , pressure P_i and gas constant R , then simple geometry shows that

$$\frac{X}{L} = \left(\frac{n}{1-n} \right) \left(\frac{\sigma_r R T_i}{P_i} \right) \quad (6)$$

so that the solutions may again be expressed in terms of n , the gas weight fraction in the explosion products. The equation of motion for the cap in the vertical h direction is

$$P - P_i = L \sigma_r \ddot{h} + L \sigma_r g + \frac{1}{2} \rho_a C_D \dot{h}^2 \quad (7)$$

where P is the general value of the pressure during the expansion. P is given by

$$P = P_i \left(\frac{X}{X+h} \right)^\gamma \quad (8)$$

initially, since the expansion is adiabatic until some small clasts begin to mix with, and supply heat to, the gas. The final term in

equation (7) represents atmospheric drag acting on the rising cap: C_D is a drag coefficient with value close to unity and ρ_a is the atmospheric density. Equation (7) is readily integrated numerically using equations (6) and (8) to yield the maximum velocity, u_t , of the products during the explosion process. The results of such calculations, and also of the use of the simpler equations (3) and (4), are summarised in Fig. 2, in which u_t is shown as a function of P_i for a range of values of n in each case.

It is likely that at least 50 wt % of the fragments ejected during the 18.10 h explosion were smaller than 3 mm (ref. 2) and able, therefore, to maintain thermal equilibrium with the gas during the period (of the order of 0.5–2 s) of acceleration of the largest clasts, implies that the velocities corresponding to equation (3) (isothermal case) may be more appropriate for vulcanian explosions than those derived from equation (4); the solutions to equation (7) certainly represent the lowest possible velocity that corresponds to any particular pair of values of n and P , as they give the greatest weight to energy losses. The speeds given by equation (4) are very close to those from equation (3) at low pressures and fall to about 70% of the equation (3) speeds as the pressure approaches 1,000 bar. The n and P values given below are means of equations (3) and (7) biased towards equation (3) on the basis of this argument.

The following combinations of initial gas pressure and released gas wt % are implied by the observed maximum velocity of 400 m s⁻¹: for $n = 30\%$, $P_i = 2$ –5 bar; for $n = 10\%$, $P_i = 10$ –30 bar; for $n = 5\%$, $P_i = 40$ –100 bar; for $n = 3\%$, $P_i = 100$ –300 bar. We argued above that the likely range of values of tensile strength of andesite at sub-solidus temperatures is 100–200 bar (refs 14, 15). Then the above permutations of pressure and gas content would imply that the explosion products of the 18.10 h event contained 2–5 wt % water. It was estimated² that equal proportions of juvenile and nonjuvenile material were expelled in the explosion, and so if it is assumed that all of the water were juvenile, 4 to 10 wt % would be required in the magma. The lower end of this range is comparable to the measured primary water contents^{21,22,28} of andesite magmas, but it is probably implied that some groundwater water was also involved.

Magma volume and conduit shape considerations

The total volume of rock expelled in the 18.10 h explosion and four other similar outbursts on the same day was about 5×10^5 m³ dense rock, approximately half of which was juvenile. We may assume that juvenile material was re-emplaced in the upper parts of the volcano between explosions, so that a total of about 2.5×10^5 m³ of pre-existing cone material was excavated.

This volume cannot have been removed from a localised region near the vent because we know² that there was little significant change in the geometry of this region during the explosive activity: if 2.5×10^5 m³ is taken to occupy a vertical cylinder with length equal to diameter, the required dimension is 68 m, which is greater than the crater bottom diameter. There is an added reason why such a geometry is unlikely. Heating of groundwater near the magma body is only possible if heat can penetrate the required distance in the time available; the repose period before the 18.10 h explosion was 50 min, and thermal waves can only travel distances of the order of a few tens of centimetres in rock in this time¹⁶.

A long, cylindrical conduit feeding the vent is the geometry which maximises the chances of groundwater being involved in the explosions. Ngauruhoe stands ~950 m above the surrounding topography; if a vertical length of, say, 500 m were involved, the excavated cylinder needed to provide both the juvenile and nonjuvenile debris for all five major explosions would have had a diameter of 32 m. Figure 3 shows the situation envisaged: a long, cylindrical conduit about 20 m in diameter containing vesicular magma of mean density ~1,500 kg m⁻³, cooled at the surface and chilled at its contact with the cone material, contains

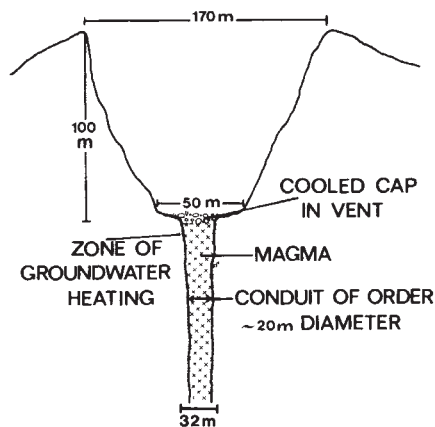


Fig. 3 Schematic of proposed cross-section through crater region of Ngauruhoe before the 18.10 h explosion.

clasts which have fallen into the conduit from earlier activity. Water in these clasts and in the cone material immediately surrounding the magma is heated to near-magmatic temperatures and, together with exsolved juvenile magmatic water, drives the explosion when the cap over the vent fails. It is not possible to estimate accurately the relative contributions from magmatic water and groundwater. The 18.10 h explosion must have removed a rock layer some 5 m thick from the conduit walls to satisfy the observed mass. Only a small fraction of this rock and its pore water could have been heated by conduction in the 50 min before the explosion, although water and steam in interconnected fissures may have been heated by convection. However, in the one to two seconds after the failure of the magma cap, that is during the period of acceleration of large blocks, rapid turbulent mixing of small fragments of disrupted magma and released groundwater would have taken place. It was shown above that up to 50% of the mass of disrupted magma could interact thermally with the evaporating water on this time scale. If all the available water in the wall rock layer were involved in the explosion, a water content of 2–3% in this rock would be consistent with the expected water contribution from the magma, as noted above.

In the above treatment we have not found it necessary to invoke the rapid mixing of juvenile material with groundwater that might lead to flash boiling²³. This mechanism has been proposed as a means of creating pressures of the order of 3–5 kbar, similar to the pressures deduced for high velocity explosions¹⁷ by applying the Bernoulli equation. Our analysis does not require such high pressures to initiate the explosion process.

Eruption column behaviour

The rise height, h , of the convective plume from the 18.10 h explosion can be computed from a formula for convective rise from an instantaneous source and compared with the observed height: h , in metres, is given²⁴ by

$$h = 1.87Q^{1/4} \quad (9)$$

where Q is the released heat energy in joules. The 18.10 h event emitted² 2×10^8 kg of rock, of which about half was juvenile (that is, hot and able to contribute to driving convection). However, about half of the material injected into the plume was lost almost immediately (after 12–15 s) by the partial collapse of the column to form pyroclastic avalanches². Thus, the heat available to drive convection is estimated to have been a maximum of 4×10^{13} J, leading to a predicted column height, h , of

4,700 m. The observed value² was at least 4,000 m above the vent, the discrepancy implying an efficiency of heat utilisation of about 50%.

This may be compared to values much closer to 100% found for maintained plumes produced by other eruptions²⁵ and also to the value found for a maintained plume at Ngauruhoe. During part of the gas-streaming eruptive phase, lasting from 13.25 to 13.45 h, prior to the series of discrete explosions, a 10 km high (above crater) convecting column existed² over Ngauruhoe. The equivalent of equation (9) for continuous column convection is^{24,25}

$$h = 8.2\dot{Q}^{1/4} \quad (10)$$

where h is the column height in metres as before and $\dot{Q}$ is the rate of release of heat energy in Watts. A 10 km column height implies a mass eruption rate of 3.2×10^6 kg s⁻¹ (ref. 25). The estimated volume² erupted in this cloud was 1.6×10^6 m³ dense rock which, averaged over 20 min and using the density of andesite equal to 2,550 kg m⁻³, corresponds to 3.4×10^6 kg s⁻¹, in good agreement with the above value, suggesting that the efficiency of conversion of heat to work was close to 80% in this case.

Vulcanian explosions are very common at the many active andesitic strato-volcanoes clustered along subducting plate margins. Some of these eruptions are more violent than those described from Ngauruhoe^{8,26}. However, previous estimates of pre-explosion over-pressures have generally been too large, and this article demonstrates that relatively small pressures are probably adequate to produce the observed behaviour during this type of eruption. Basaltic andesite magmas have water contents comparable to those deduced for the explosions^{21,22,28}, and the involvement of groundwater is possibly a significant feature of the events, but not an essential feature as proposed by Schminke²⁷. It is not necessary to invoke violent mixing of juvenile material and groundwater on a large scale to explain the explosions though such mixing on a small scale may be a significant feature of some vulcanian explosions. Eruption cloud heights can be related to the rate of release of juvenile material during steady gas-streaming activity, and to the amount of released material in discrete explosions, using the formulae found to apply to larger-scale eruptions²⁵.

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Concentrations of metals in very small volumes of soil solution

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A new method of sampling very small amounts of soil solution (0.3 g) shows that soil solutions contain high concentrations and unusual proportions of metals. In the soils studied, the solution is close in both metal proportions and total metal mass to what may be taken up annually by the growth of plants at the sites sampled. Composition of soil solution varies seasonally and with depth in soil.

A FUNDAMENTAL problem in soil science and plant nutrition has been the determination of the true chemical composition of the films of solution which surround particles of normal, unsaturated soils and which are in contact with the roots of growing plants. Attempts to determine metal concentrations in soil solution have been hampered because conventional analysis requires large amounts of solution, and the collection of large samples generally alters the composition of the solution¹.

A method for sampling very small amounts of solution from undisturbed, unsaturated soils in the field was used for this study, and techniques originally used in nuclear geochemistry have been used to analyse the samples. This has the advantage that much smaller samples (roughly single-drop size) may be analysed to greater accuracy for both major metals and related trace metals than has previously been possible.

Field selection and sampling method

The soil solution is rapidly blotted onto a stack of specially cleaned absorbent paper. In the field, the sampler cleared a flat space at an appropriate horizon in the soil, and placed a porous-plastic (Nuclepore) filter on the soil surface. Then, wearing an acid-cleaned polyethylene glove, the sampler pressed with his thumb a stack of six specially cleaned and weighed 4-cm filter paper disks tightly against the plastic filter for a few seconds. The stack was put into a clean Teflon bottle and tightly sealed for reweighing in the laboratory. Rare contamination of the solution samples with particles from the soil was revealed by easily visible brown staining of the white absorbent paper, and proved in all cases to be associated with rupture of the plastic filter. The few such samples were discarded.

Two published reports^{2,3} have proposed this sampling method in principle, and results have been reported by Schuffelen *et al.*⁴ of a study using a similar sample collection method.

The study site, Thompson Canyon, comprised an area of 12.2 km² in the northern part of Yosemite National Park in the central crest region of the Sierra Nevada of California at an altitude of ~2,900 m (meadow surface). The area was selected because of freedom from disturbance by human activity, uniformity of rock (quartz monzonite) which has provided material to the soil since glaciation 10,000 yr ago, the fact that all water entering the watershed comes from precipitation, and the rather simple and stable assemblage of plant and animal species. The watershed has been used since 1970 for studies of geological and biological fractionation of metal families in terrestrial food chains⁵⁻⁹.

Methods of analysis and soil sampling

Metals determined in the samples were the alkali metals K, Rb and Cs and the alkaline earth metals Ca, Sr and Ba. These metals were selected because the members of each family exhibit a narrow but definite range of behaviour in nature owing to similar electronic properties and varying ionic size. The metals have widely varying geochemical abundances, and most have been used extensively in soil science investigations. Na and Mg were not suited to the same analytical techniques as the six metals reported in this study.

Stable isotope dilution mass spectrometric analysis was used for all determinations. It is the most sensitive and accurate method known for determining very small amounts of many metals; the extremely small samples (~0.3 g solution) collected in this study were readily analysed for the six metals using this technique. The most extensive development and application of the technique has been in the field of nuclear geochemistry, and its use in this study is possible largely through its refinement for analysis of lunar material¹⁰. A known amount of isotopically enriched tracer was equilibrated with the sample so that the resultant isotopic ratio was a mass-weighted average of the isotopic composition of the enriched tracer and the natural isotopic composition of the sample. This resultant ratio was measured on a solid-source, thermal-emission mass spectrometer, and the sample mass calculated.

Seventeen samples of soil solution were analysed. These were taken from three localities (total of five horizons). Soils selected (entisols) were (1) a humus-rich soil in the centre of the broad, deep meadow in the bottom of the glacial valley, sampled in a single near-surface zone containing sedge roots; (2) a soil (humus-rich near the surface but less deeply developed than the meadow soil) perched on a narrow hillslope terrace, from which solution was sampled at both 5-cm (growth zone of shallow-rooted herb and sedge plants) and 30-cm depths (zone of deeper rooted plants such as large lupine); (3) a soil on the boundary of

Table 1 Metals in mid-meadow soil-solutions compared to phreatic water, stream water, and average snow (parts per 10⁹)

Metal	4 mid-meadow soil-solutions	Phreatic water, same locality	Stream water, same locality	Snow*
K	7,000	11.0	62.0	9.7
Rb	71	0.17	0.26	0.042
Cs	11	0.005	0.011	0.0048
Ca	1,740	530	380	18
Sr	32	13	7.4	0.15
Ba	64	6.5	1.2	0.22
K/Rb	98	66	240	230
K/Cs	630	2,300	5,600	2,020
Ca/Sr	54	41	52	109
Ca/Ba	27	82	310	82
K/Ca	4	0.022	0.16	1.8

*Vertically integrated snowpack at maximum depth, 90% of total annual precipitation.

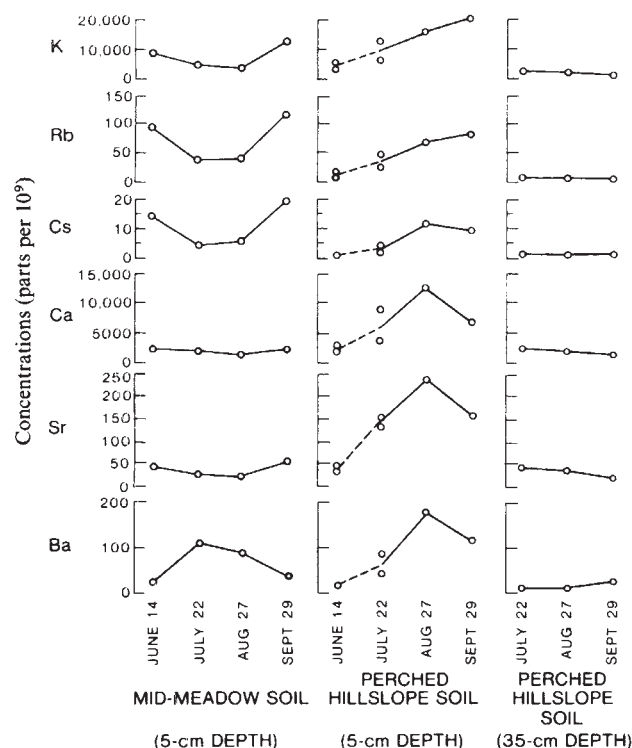


Fig. 1 Variation through the growing season in the metals composition of soil solutions: well developed soils in mid-meadow and on a hillslope platform (concentrations in parts per 10^9).

conifer forest and meadow, supporting both conifers and meadow plants.

For sample preparation the filter disks were leached of sample using 20 ml of 0.1 M, highest purity HCl for several hours at 55 °C in an acid-cleaned quartz centrifuge tube and then centrifuged to precipitate fibres. Aliquots of this solution were removed with a pipette, isotopically spiked, and analysed for metals.

Tracers for all six metals were mixed together and added to aliquots of solutions made up from soil solution samples. The six metals were analysed in the mass spectrometer without chemical separation. Actual metal masses that were determined ranged from 160×10^{-9} g for K to 0.02×10^{-9} g for Cs, with blanks always less than 10% of these masses. To minimise contamination, special precautions were taken; these involved clean rooms, ultra-pure reagents, ultra-clean apparatus, and low contamination procedures in the field¹¹.

The accuracy and precision of the soil solution sampling and analytical method were tested using a homogeneous stock solution containing metals in concentrations very similar to those of the soil solution. The stock solution was sampled in two different ways. In one, an aliquot was taken directly from the bottle, spiked, and analysed with minimum treatment. In the second, a

sample was blotted onto cleaned filter paper through a Nuclepore plastic filter and removed by leaching, a method analogous to the method of sampling the soil solution in the field. The data (not presented here) show that passage of moisture through the plastic filter and subsequent acid extraction from the absorbent paper do not introduce any large errors: results obtained using the two ways of sampling the known stock solution agreed within a few per cent.

In addition, reproducibility of results from actual field samples was tested by two methods. (1) Two samples of soil solution were taken in rapid succession directly from the cleared soil surface in the field, only a few centimetres apart (results plotted as double data points in Fig. 1, 14 June collection, perched hillslope soil; Cs and Ba analyses failed on one of the samples). At the same site, a soil sample (1,500 g) was thoroughly stirred in a clean polyethylene pan and two solution samples were collected from it in rapid succession (plotted as double points in Fig. 1, 22 July collection). As shown in Fig. 1 the variations in the duplicates are important fractions of the total masses, especially for Ca, but generally both values in each duplicate pair are close, even for Ca, and both fit well within the seasonal trend plotted. The variation must be attributed to inhomogeneities in the soil on a scale of a very few centimetres.

In a primitive version of the method used in this study¹² the bottom disk of the stack of absorbent papers was in direct contact with the soil and was discarded. This method introduced errors because the heavier alkaline earth metal ions (Sr and Ba) were preferentially retained in the bottom disks of the stack, through which the sample first passed. This finding led to the use of the porous plastic filter between soil and absorber during collection.

To clean the absorbent paper the Whatman No. 40 ashless, cellulose-acetate filter paper disks (5.5 cm) were batch-cleaned by leaching for 24 h in each of three successive 55 °C baths of 25% HCl solutions of increasing purity (analytical reagent grade, G. Frederick Smith distilled, National Bureau of Standards distilled). Moderately thorough water rinses (to pH 4 or 5) were used between each acid bath; highest purity rinse water was sucked through a few disks at a time on a Buchner funnel. The disks were vacuum dried between each acid bath. The series of HCl leachings was followed by a similar series in 0.5% highest purity HF and in 1% NH_4Cl at pH 7. The final rinse, after the NH_4Cl , was very sparing, so that some NH_4^+ would be left on the paper (E. E. Dickey, personal communication). The 0.4 μm pore size Nuclepore filters were cleaned in the same way as the papers. The HF and NH_4Cl steps reduce by 10-fold a troublesome Ba analytical blank, compared to that for a simple HCl sequence¹².

Results and discussion

Compositions of soil solution are quite different from those of any phreatic and stream waters in the watershed and elsewhere¹³⁻¹⁵. Metal concentrations in soil solution samples taken near the soil surface were as much as 1,000 times higher than those in phreatic water or stream samples collected at the same location (Table 1). Soil solution is the only type of water in the

Table 2 Moisture content of soils from which solution was sampled

Date	Mid-meadow soil (5 cm depth) (19% organic)	Perched hillslope soil		Forest-meadow boundary soil	
		(5 cm depth) (7.1% organic)	(30 cm depth) (2.7% organic)	(5 cm depth) (6.3% organic)	(30 cm depth) (3.3% organic)
14 June	1.14	1.05	—	0.96	0.61
22 July	ND	0.92	0.60	—	—
27 Aug.	0.98	ND	ND	—	—
29 Sept.	1.05	0.98	0.63	1.00	0.58

Values represent mass of moisture as fraction of mass of air-dry soil. ND, not determined; —, no sample collected.

Table 3 Comparison of metal availability in a meadow soil* by two different measures

Metal	Exchangeable bases† (mEq per 100 g)	In soil solution‡ (mEq per 100 g)
Na	1.3	—
K	0.11	0.0033
Ca	0.95	0.0016
Mg	0.23	—
Total exchange cations	21	—
K/Ca	0.12	2.1

*This soil (49% water) and soil solution collected in 1973.

† Exchangeable bases measured with NH_4^+ at pH 7 by US Soil Conservation Service, Riverside, California.

‡ Blotting method of this study.

watershed that has higher concentrations of alkali metals than alkaline earth metals. Stream water, phreatic water, and snow all contain more alkaline earths than alkalis. In no other natural waters measured are the concentrations of Rb and Cs, either absolutely or relative to K or to the alkaline earth metals, as large as they are in soil solution.

Solutions were collected at various times in the summer, at various soil depths, and from various soil types. The data are plotted in Fig. 1. In solutions near the surface (5 cm depth) of humus-rich soils, the concentrations of alkali metals (K, Rb, Cs) are highest in the autumn (mid-meadow and perched hillslope soils). In samples taken at greater depth below surface (30 cm, more mineralic zones), the alkali concentrations are lowest in the autumn (perched soil). For these deeper zones the seasonal trend appears less distinct in Fig. 1 only because the absolute concentrations and their changes are smaller, and because the scale of the plot has not been expanded.

Three possible factors influencing the seasonal soil-solution composition are the following: (1) metal uptake and release of metals by the standing crop of plants; (2) through-flow of solutions having different metal contents, and (3) uptake and release of metals by humus and clay in soils, in response to temperature, pH, or other physical and chemical factors. As for the effects due to the growth of plants, the mass of metals in soil solutions above the water table in mid-meadow is comparable to the mass of metals in the leaves of the standing crop of sedge (Table 4). Metal ratios in sedge leaves vary strongly with time of season¹⁶, but these variations may represent only internal redistribution of metals between the leaves and the root systems of these plants. Variation in soil-solution composition caused by through-flow of soil solutions of variable composition is unlikely in these poorly drained soils, except in the earliest snow-melt part of the season. pH and temperature may affect the release of metals from humus and clay; pH was between 4 and 5 at all times of the season for all soils and average soil temperatures can rise

measurably in mid-season. Equilibration times needed to replace metals in soil solution from the humus and clay reservoirs have not been determined for the soils of this study.

Apart from seasonal variations, the major differences between the soil solutions collected near the soil surface (humus-rich zones) and those collected at greater depths (mineralic zones) are the following: (1) the metal concentrations are generally higher in the near-surface samples, and (2) alkali metals are more abundant relative to the alkaline earth metals in the near-surface samples. K/Ca ratios are most commonly greater than unity in the deeper zones. It is empirically true in samples from the field area of this study that, in direct correspondence to the composition of the shallow and deep soil solution, the leaves of shallow-rooted plants (sedges) have higher K/Ca ratios than those of more deeply rooted plants (conifers)¹⁶. Roots of these plants were present in the respective zones at the actual sampling sites.

The moisture contents of the soils sampled in this study (Table 2) are high and do not vary greatly. There are distinct differences between shallow, organic-rich soils and deeper, mineralic soils, but these subalpine soils do not dry out through the summer as do soils in other environments. The effect of lowered moisture content on the absolute or relative concentrations of the two families of metals determined are not known from the experiments of this study; it is accepted that changes in moisture content alter the distribution of monovalent and divalent metals between soil and solution¹⁷, but it seems that the seasonal variation in solution metal composition observed in this study (Fig. 1) is to be associated with other factors. It is not known whether samples large enough for analysis could be collected by the blotting technique from soils much less moist than those of this field area.

The data presented in Table 3 show that in the particular non-agricultural soils of the study area the reservoir of metals contained in soil solution is much smaller than the reservoir available as exchangeable cations. In the study area the mass of metals in the soil solution is approximately equivalent to the mass turned over by plants in the annual growth cycle (Table 4). The proportions of the metals in the solution reservoir are very different from those in the exchangeable cation reservoir: in soil solution, the alkali metals seem to be relatively more abundant than the alkaline earth metals, whereas in the exchangeable cation reservoir the reverse is true (Table 3, K/Ca values 2.1 and 0.12, respectively).

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Table 4 Comparison of masses of metals in soil solution and in annual plant productivity

Metal	In soil solution	In annual plant productivity
K	7,000	14,000
Rb	71	81
Cs	11	8.5
Ca	11,750	5,200
Sr	32	96
Ba	64	81

Mid-meadow soil; mass of metals in solution to 10 cm depth; plant values from standing crops of sedge leaves; from unpublished data of Patterson and Elias; data in g per hectare.

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Crystal structures of *Streptomyces subtilisin* inhibitor and its complex with subtilisin BPN'

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Crystal structure of a microbial protein proteinase inhibitor SSI (Streptomyces subtilisin inhibitor) and its complex with subtilisin BPN' were solved at 2.6 Å and 4.3 Å resolution respectively. The mode of binding to the proteinase of SSI is compared with that of bovine pancreatic trypsin inhibitor, soybean trypsin inhibitor and the substrates. Stereochemical considerations for the possible evolutionary relationship between SSI and pancreatic secretory trypsin inhibitor are presented.

MANY protein proteinase inhibitors from animal and plant tissues have been extensively studied¹. However, bovine basic pancreatic trypsin inhibitor²⁻⁴ (Kunitz, BPTI) and soybean trypsin inhibitor^{5,6} (Kunitz, STI) are the only protein proteinase inhibitors which have been studied by X-ray diffraction. *Streptomyces subtilisin inhibitor* (SSI), isolated from *Streptomyces albogriseolus* by Murao and Sato^{7,8}, is one of the few well characterised microbial protein proteinase inhibitors. SSI has been characterised chemically and spectroscopically and by the X-ray diffraction studies carried out in a collaborative study involving nine laboratories, led by K. Hiromi (summarised in ref. 9). Unlike classical trypsin inhibitors¹, SSI strongly ($K_1 = 10^{-10}$ M)¹⁰ and specifically⁸ inhibits bacterial alkaline proteinases (such as subtilisin BPN'), although it also weakly inhibits α -chymotrypsin and trypsin ($K_1 = 10^{-6}$ and 10^{-4} M, respectively)^{11,12}. SSI is a stable dimer¹³ (I_2) composed of two identical subunits each of molecular weight 11,483 (ref. 14). In the trigonal crystals (P3₁21, $a = b = 40.6$ Å, $c = 115.2$ Å) of SSI analysed here, the two subunits are related by a crystallographic diad¹⁵.

The inhibition of subtilisin BPN' by SSI occurs when one subunit (I) of SSI inhibits one molecule (E) of subtilisin^{16,17}, and the molecular species of the SSI · subtilisin complex in solution was characterised as E_2I_2 (ref. 18). The orthorhombic crystal of this complex (I222, $a = 77.5$, $b = 186.9$, $c = 69.6$ Å), analysed here, contains half the complex molecule (EI, MW ~ 39,300) in an asymmetrical unit¹⁵. Furthermore, the amino acid sequence of the subunit of SSI, a single polypeptide chain with two disulphide bridges, was determined by Ikenaka *et al.*¹⁴. Chemical evidence, including the unusual susceptibility of the Met-73-Val-74 bond to trypsin and α -chymotrypsin, led these workers to propose that this was the susceptible bond¹⁴. We have shown here that this hypothesis is correct by crystal structure analysis of the complex molecule.

Structure of SSI

The structure analysis of SSI at 4 Å resolution was reported previously¹⁹. For 2.3-Å resolution study, about 13,000

reflections (including Friedel pairs) were collected for each of the three heavy-atom derivatives, $(NH_4)_2Hg(SCN)_4$, $(NH_4)_2Pt(NO_3)_4$ and ethylmercuric thiosalicylate, for which the root-mean-square (r.m.s.) heavy-atom structure factor/r.m.s. lack-of-closure error was 38.6/19.3; 34.9/18.6; and 36.8/19.6 electrons, respectively. Detailed analysis of the statistics, however, revealed that the phasing between the 2.3-Å and 2.6-Å spheres was unreliable so that the maximum resolution of the map was 2.6 Å. The map was interpreted using a half-mirror device²⁰ and the 1 Å = 2 cm Kendrew model. For rapid measurement of the coordinates of the wire model, a semi-automatic device using two He-Ne laser tubes and magnetic encoders was developed²¹. Details of the structure analysis will be published elsewhere²².

A schematic drawing of the subunit has been previously presented²³; the electron densities are easily interpretable except for the N-terminal four residues (almost missing) and the region Ala-62-Met-70, where only the approximate course of the main chain can be traced. The size of the subunit is about $25 \times 35 \times 40$ Å, whereas that of the dimer shown in Fig. 1 is about $30 \times 40 \times 65$ Å. The subunit has a five-fold antiparallel β -sheet twisted as usual²⁴, and two short α -helices (α_1 (46-57) and α_2 (100-107)). The topology of the β -sheet in relation to the primary sequence is such that the five β -strands are arranged from the top to the bottom of the molecule thus: β_5 (residue numbers 89-97)↑, β_4 (77-87)↓, β_1 (10-19)↑, β_2 (29-35)↓, and β_3 (40-42)↑, where arrows indicate the polarity relationship between the polypeptide chains. The same β -sheet topology is seen elsewhere only as part of the variable region of immunoglobulin^{25,26}, in which the 30-40 β -strand corresponds to the β_1 strand of SSI.

According to Levitt and Chothia²⁷, SSI subunit belongs to the "class II" or "all β " type of proteins. Most of the non-crystallographic predictions⁹ on the states of some specific amino acid residues have proved qualitatively correct. These include the nature of the peptide NH (refs 28,29), conformation of S-S bridges (M. Nakanishi, unpublished observation), and the states of tryptophyl^{30,31}, tyrosyl^{30,32}, histidyl^{32,33}, and methionyl residues³². Detailed evaluation of these results will be published elsewhere²².

The subunit-subunit interface

The interface is formed by the stacking of a β -sheet of one subunit on top of a β -sheet of the other subunit, related by a diad. The planes of the two β -sheets are roughly parallel (Fig. 1a), whereas the β -strands in the opposite sheets are inclined at about 30° to each other (Fig. 1b). Similar stacking of β -sheets at the subunit-subunit interface has been previously reported only at the dimer-dimer interface of concanavalin³⁴ and in the monomer-monomer interface of glyceraldehyde-3-phosphate dehydrogenase related by the 'P' axis³⁵. In the former case, the β -strands in the opposite sheets are inclined to each other in about the same way as in SSI, but in the latter case, they seem to

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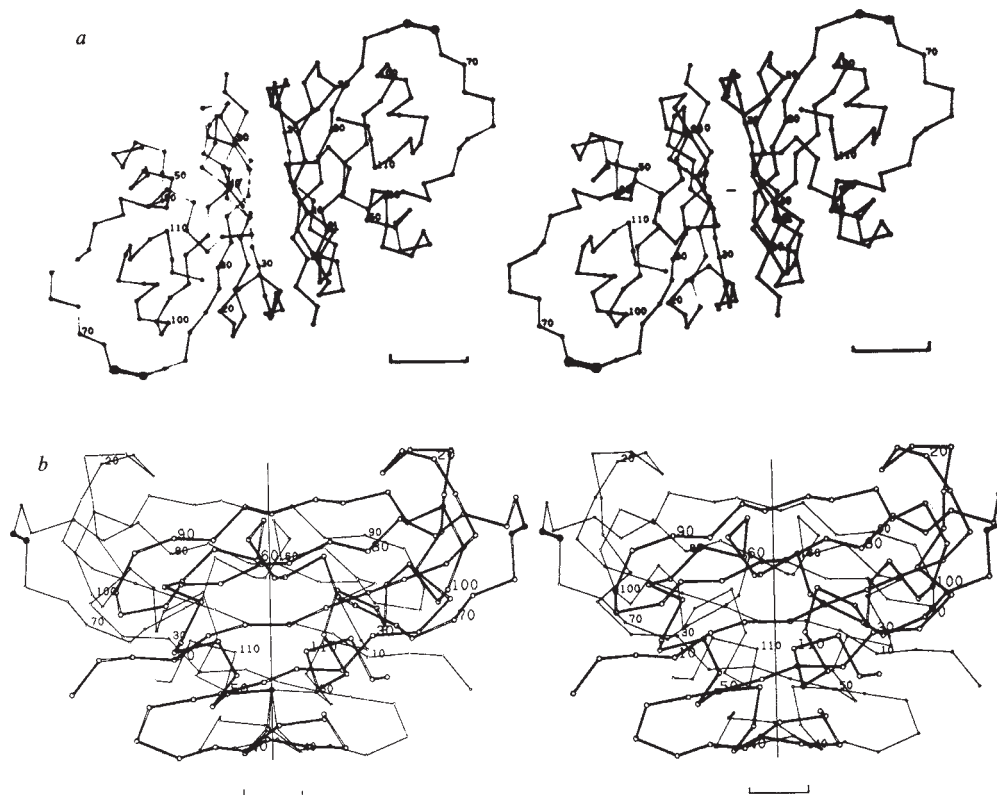


Fig. 1 α -Carbon chains of a SSI dimer viewed *a*, along the diad and, *b*, perpendicular to the diad. The reactive site PI, PI' residues are marked. Scale bars represent 10 Å and 5 Å, respectively, for *a* and *b*.

run almost parallel. All the 48 intersubunit contacts of less than 3.8 Å, except for three possible hydrogen bonds, are non-polar in agreement with the observed susceptibility of the dimer to SDS (ref. 36). An interesting feature of the side chain packing in the interface is the straight side chain of Arg-90 held tightly in a pocket formed by Thr-15, Ala-22, Pro-27 and Leu-79 of the opposite subunit, and constituting an effective device for correct alignment of the subunits.

Of the 15 residues participating in the subunit-subunit interaction, only one replacement residue, Thr-34 to Asn, is seen in the closely related dimeric inhibitor, plasminostreptin (isolated from *Streptomyces antifibrinolyticus*)³⁷⁻³⁹, which has 64% overall homology with SSI. This suggests that the purpose of the dimeric structure is the preservation of its own function and or stability. Inspection of the model shows that all the amino acid side chains replaced in plasminostreptin can be accommodated in the structure of SSI. SSI and plasminostreptin are, therefore, homologous proteins.

The reactive site and the C-terminal region

The electron density around the reactive site residues (Cys-71 to Tyr-75) is very high indicating the rigidity of this region as in BPTI⁴ and STI⁶. The region seems to be tightly held by the β_4 -strand embedded in the β -sheet and by the 71-101 disulphide bridge which, in turn, is fixed to the core of the subunit through helix α_2 and the C-terminal residues. The C-terminal four residues Val-110, Phe-111, Ala-112 and Phe-113 (the 111 and 112 order has been reversed (T. Ikenaka, personal communication)), can be selectively removed from intact SSI by treatment with carboxypeptidase A (M. Sakai, M. Odani & T. Ikenaka, in preparation). This derivative, SSI-A, still weakly inhibits subtilisin BPN'. However, the inhibition is only temporary if an excess of the enzyme is present. The normal inhibitory activity was almost completely recovered by adding a 30-fold excess of the C-terminal deca-, nona- or octapeptides to SSI-A. These observations, together with the UV difference

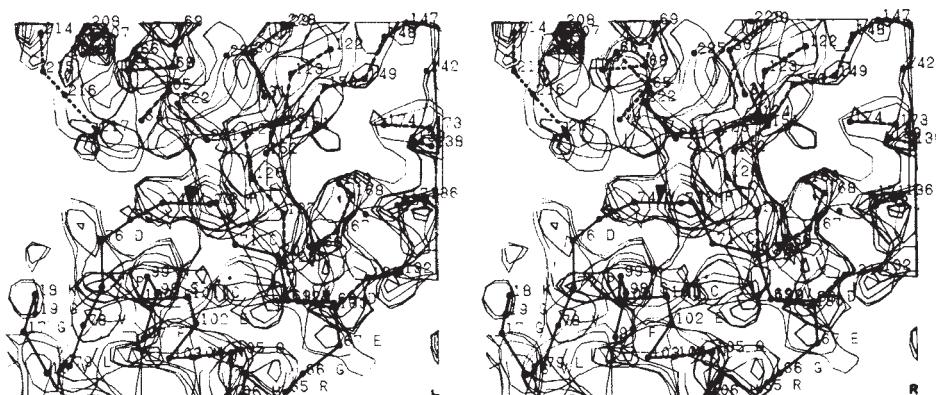


Fig. 2 α -Carbon chains of SSI (solid lines) and subtilisin BPN' (broken lines) in the region of contact fitted into a 4.3-Å resolution electron density map of the SSI-subtilisin complex. The scissible bond is marked by ∇ .

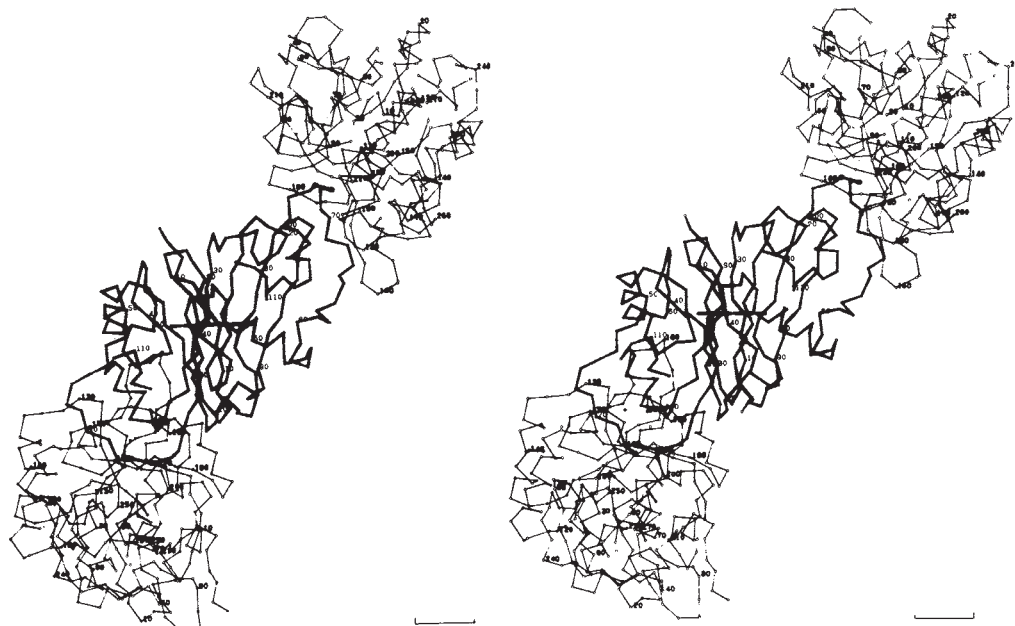


Fig. 3 α -Carbon chains of a complex of SSI dimer (solid bonds) with two subtilisin BPN' (open bonds). The diad (horizontal line in the centre) is inclined to the plane of the paper at an angle of 60° . Scale bar represents 10 Å.

spectra, suggested that SSI-A has a somewhat loosened structure which is susceptible to proteolytic attack, but that the structure of intact SSI is almost fully restored on addition of the C-terminal oligopeptides. The C-terminal four residues make 43 inter-atomic contacts of less than 4 Å with the rest of the molecule, 93% of them being non-polar; this may explain the destabilisation of the molecule when they are removed.

Comparison of main-chain conformations of the reactive site residues and the substrate

Table 1 compares the main-chain torsion angles in the reactive sites of BPTI, STI, SSI and a hypothetical substrate⁴⁰ when bound to subtilisin BPN'. For BPTI and STI the values were obtained from the published refined coordinates^{3,6}. The values for SSI, although not yet refined, are probably correct within 15° . The values for the substrate are based on provisional coordinates. Between BPTI and SSI, the angles for P_2 , P_1 , P_1' and P_2' agree within experimental error, despite the completely different amino acid sequences at the reactive site and the different proteinase specificity (BPTI does not inhibit subtilisin BPN'). For P_2 and P_1 the agreement extends to STI as well. The

difference in P_2' parameters between STI and SSI clearly reflects different chain conformation. The P_1' parameters of STI present a problem. The combination of ϕ and ψ is energetically unfavourable, and Sweet *et al.*⁶ noticed that, in the given orientation of the peptide bond between Ile-64' and Arg-65', there was no suitable hydrogen bond for NH(65'). Re-examination of the map shows that $\phi(P_1')$ may well be $\sim 180^\circ$ (J. Janin, personal communication). For P_3 , the trypsin inhibitors do not share a common conformation with SSI. This reflects the fact that, in the trypsin inhibitors, P_4 , P_5 , ----- are not on the surface of the enzyme, but those in SSI are still on the surface.

Comparing SSI and the hypothetical substrate, the overall agreement (except for $\psi(P_2')$) is clear, if allowances are made for the appreciably large errors in the latter coordinates. The difference in $\psi(P_1)$ and $\phi(P_1')$ might be significant, but detailed study must await a high resolution map of the SSI · subtilisin complex. At the resolution now obtainable, there is no clear indication of any change in SSI conformation on complex formation. The structure of the hypothetical substrate was deduced from X-ray difference Fourier studies on the binding of various peptide chloromethyl ketones⁴¹ and polypeptides⁴⁰ to subtilisin BPN'. Apparently because of weak P_1 specificity (compared to trypsin), the results were controversial. Despite these difficulties, the present comparison strongly suggests that the productive binding mode of substrates to subtilisin proposed

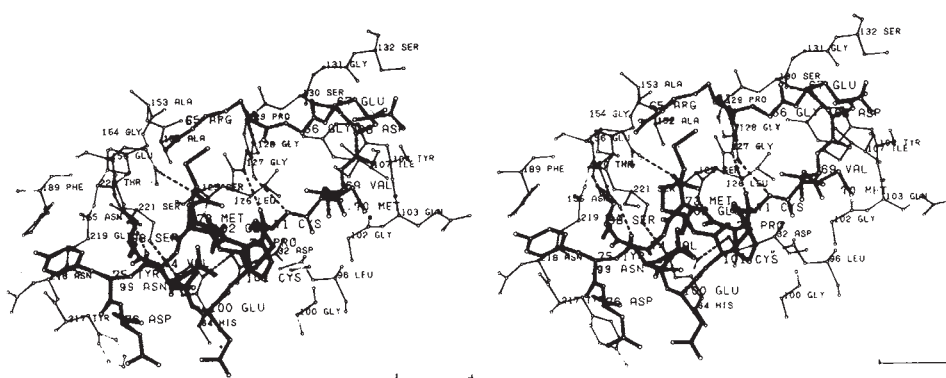


Fig. 4 The region of contact of a model of the SSI (in solid bonds and larger characters) and subtilisin BPN' (in open bonds)⁴³ complex constructed as for Fig. 3. All residues relating to the intermolecular short contacts of less than 4 Å are shown. The bonds for Arg-65'-Met-70' are striped because the corresponding electron density in the SSI crystal is very weak. Scale bar represents 5 Å.

by Kraut *et al.*⁴⁰ is essentially correct and that, as in the case of trypsin inhibitors^{3,6}, the binding mode of a protein proteinase inhibitor (SSI) to subtilisin is, at least, closely related to that of substrates.

Comparison of overall conformation in BPTI, STI and SSI

The similar conformations of the reactive sites of the three inhibitors prompted us to investigate whether the similarity extends to any other part of the molecule. Despite the large difference in length (BPTI, STI and SSI have 58, 181 and (2×)113 residues, respectively) and apparent heterology in primary sequences, this seemed relevant, as three-dimensional structure is one of the properties which is probably most highly conserved during evolution. The coordinate systems of BPTI and STI were translated and rotated by a least-squares procedure to optimise the overlap between the corresponding main-chain atoms of the reactive site residues (P_2 to P'_2 for BPTI, P_2 to P'_1 for STI) and those of SSI. The general non-relatedness

hypothetical substrate (Table 1), and then adjusted by fitting it to the electron density through computer graphics (Fig. 2). At this resolution, there is no sign of major conformational change on complex formation in either SSI or subtilisin BPN'. As in the case of trypsin inhibitors^{3,6}, the region of contact is small: of the 113 and 275 amino acid residues, respectively, composing SSI subunit and subtilisin BPN', only 11 and 19 residues are in contact. Recent estimation of the thermodynamic parameters of the complex formation using the temperature dependence of K_1 yielded a value $\Delta H = +4.2 \text{ kcal mol}^{-1}$ and $\Delta S = +57 \text{ e.u.}$ (at 25°C , $\text{pH } 7.0$)¹⁰, showing that the process is entropy driven as is the case for other trypsin inhibitors⁵.

The SSI-subtilisin interface

Figure 4 was obtained by the same procedure as for Fig. 3. Because the parameters defining relative translation and orientation of the two molecules were adjusted with reference to the electron density map, the nature of the model is semi-empirical.

Table 1 Reactive site residues and main-chain conformations of BPTI³, STI⁶ and SSI

	P_3		P_2		P_1		P'_1		P'_2	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
BPTI	-86	-28	-64	152	-116	87	-135	166	-116	87
STI	-46	-21	-77	136	-89	85	(-135)	(-115)	-175	-179
SSI	-123	141	-52	134	-92	89	-117	169	-120	78
Hypothetical substrate	(-157)	(172)	(-84)	(162)	(-116)	(26)	(-62)	(136)	(-112)	(-70)
BPTI	Pro		Cys		Lys(15)		Ala(16)		Arg	
STI	Ser		Tyr		Arg(63)		Ile(64)		Arg	
SSI	Cys		Pro		Met(73)		Val(74)		Tyr	
Hypothetical substrate	Ala		Ala		Tyr		Val		Val	

Corresponding data (in parentheses) for a hypothetical substrate⁴⁰ productively bound to subtilisin BPN' are only provisional. Dihedral angles within 35° of the corresponding ones in SSI are outlined.

between SSI and BPTI (or STI) was clear, although there is some topological resemblance between residues 6–43 of BPTI and residues 60–103 of SSI (details will be published elsewhere)²². Most probably, the three inhibitors have evolved convergently from independent ancestral proteins, for the common purpose of inhibiting serine proteinases, and resulting in a similarity of the reactive site alone.

It is interesting that there are already three distinct patterns of 'protein proteinase inhibitor fold', and the number of such folds is likely to increase, as there are many examples (such as soybean Bowman-Birk inhibitor) of compounds whose structure cannot be similar to any of the known folds, because they have a different pattern of disulphide bridging⁴². In contrast, the serine proteinases, the target molecules, show only two basic patterns of chain folding, those of trypsin and subtilisin.

Structure of the complex molecule

The mixture (~1:2) of SSI and purified subtilisin BPN'(EC3.4.21.14) (Nagase, lot. 7370935) in 0.05 M Tris-HCl buffer ($\text{pH } 7.5$) was separated from excess inhibitors and auto-digested enzymes by gel filtration. The crystals were easily obtained by leaving the main fractions in a cold room¹⁶. The structure was solved at $4.3 \text{ \AA}$ resolution using two heavy-atom derivatives, $(\text{NH}_4)_2\text{Pt}(\text{NO}_2)_4$ and K_2PtCl_4 , for which the ratio r.m.s. heavy-atom structure factor/r.m.s. lack-of-closure error for the 3,550 independent reflections was 16.4/7.6 and 15.7/7.3, respectively, and of which details will be published elsewhere.

The model shown in Fig. 3 was first constructed using a rough correspondence in reactive site conformation between SSI and a

The model is based on the coordinates determined for the crystals of SSI alone and subtilisin BPN' alone^{43,44}. Thus, in the actual structure, induced fit could cause movement of, for example, Tyr-104 of the enzyme (as observed in the difference Fourier studies^{40,41} on subtilisin BPN'). As in trypsin inhibitors^{3,6}, the sequence of P_4 (Met-70', (prime indicates the inhibitor)), P_3 (Cys-71'), P_2 (Pro-72') and P_1 (Met-73') makes an antiparallel β -sheet with the Ser-125–Gly-127 sequence of the enzyme. A possible scheme for hydrogen bonding is shown. Discussion of the nature of the interaction between the susceptible bond and the active serine (Ser-221) must await high resolution studies.

There are two kinds of interaction which differ from those made by trypsin inhibitors^{3,6}. First, P_4 (Met-70'), P_6 (Asp-68') and P_7 (Glu-67') are still on the surface of the enzyme. Although the precise mode of interaction involving these residues cannot yet be considered because of their flexibility in the SSI crystal, the proximity of P_4 to Gly-102, Glu-103 and Tyr-104 and of P_6 and P_7 to Ser-130 is clear. In the complex crystals, the electron density for the region Glu-67' to Met-70' is comparable with that for other regions (Fig. 2). We may tentatively conclude that the flexible loop (Glu-67'–Met-70') of SSI becomes fixed on complex formation with subtilisin BPN'. We suggest that the P_4 and the subsequent (to the N terminus) residues may be important for the interaction of substrates with subtilisin BPN'. Robertus *et al.*⁴⁰ suggested that the S_4 site has a strong affinity for the phenylalanine side chain and that the site might have an important role in determining the cleavage point for the polypeptide substrate. P'_3 (Asp-76') and the subsequent (to the C terminus) residues, on the other hand, are not in contact with the enzyme.

in the complex of BPTI with trypsin^{3,4}. In this case, the residues Gly-36', Gly-37' and Cys-38', which are close to the reactive site through the 14-38 disulphide bridge, are on the surface of the enzyme. A hydrogen bond is present between the peptide carboxyl group of Gly-37' and the phenolic hydroxyl group of Tyr-94 (ref. 3). However, optimal superposition of BPTI on SSI (mentioned above) shows that the stereochemical relationship of the secondary site to the primary site is completely different

Table 2 Comparison of amino acid sequences of SSI¹⁴ and plasminostreptin³⁷ with those of the PSTI family

^a Homology(%)	0	0	23	2	20	59	100	82	62	95	56	28	90	100	0	0	37	0	100	64	0	0
Residue no. (SSI)	65	66	67	(P6)	(P5)	P4	P3	P2	P1	P1'	P2'	(P3')	77	78	79	80	81	82	83	84	85	86
SSI	R	G	E	D	V	V	C	T	K	V	Y	D	P	V	L	L	T	V	D	G	V	W
Plasminostreptin	V	R	G	D	V	V	C	T	K	V	Y	D	P	V	L	L	T	V	D	G	V	W
Acrosine inhibitor	—	—	H	L	F	F	C	T	R	Q	M	D	P	I	C	G	T	—	N	G	—	—
(porcine)	—	—	—	—	—	V	C	T	K	E	L	H	R	V	C	G	S	—	D	G	—	—
Bdellin 3(leech)	—	—	—	—	—	V	C	T	K	E	L	H	R	V	C	G	S	—	D	G	—	—
PSTI	—	—	E	V	N	G	C	P	R	I	Y	N	P	V	C	G	T	—	D	G	—	—
(bovine)	—	—	E	V	N	G	C	P	R	I	Y	N	P	V	C	G	T	—	D	G	—	—
(ovine)	—	—	E	V	N	G	C	P	R	I	Y	N	P	V	C	G	T	—	D	G	—	—
(porcine)	—	—	E	V	S	G	C	P	R	I	Y	N	P	V	C	G	T	—	D	G	—	—
(human)	—	—	E	L	N	G	C	T	K	I	Y	N	P	V	C	G	T	—	D	G	—	—
Submandibular inhibitor	—	—	—	—	—	A	C	P	R	L	H	E	P	I	C	G	T	—	D	H	—	—
I (canine)	—	—	—	—	—	M	C	T	M	D	Y	R	P	L	C	G	S	—	D	G	—	—
II (canine)	—	—	—	—	—	A	C	T	K	E	Y	R	P	L	C	G	S	—	D	N	—	—
Avian ovomucoid inhibitor	(17)	(17)	(17)	(18)	(17)	(17)	(31)	(20)	(7)	(21)	(16)	(18)	(28)	(16)	(31)	(31)	(18)	(31)	(31)	(15)	(31)	(31)
G	(14)	(14)	(8)	(9)	(8)	(7)	(31)	(4)	(7)	(8)	(10)	(3)	(2)	(9)	(31)	(31)	(12)	(31)	(31)	(13)	(31)	(31)
K	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
D	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
V	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
E	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
F	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
N	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
S	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
T	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
R	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Q	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
P	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
C	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
M	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
L	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
H	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
A	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
I	(1)	(1)	(3)	(2)	(3)	(5)	(1)	(3)	(6)	(2)	(3)	(3)	(1)	(6)	(1)	(1)	(1)	(1)	(1)	(2)	(1)	(1)
Y	(1)	(1)	(3)	(2)	(3)																	

The comparison is made for *a*, the primary contact region and *b*, the secondary contact region (see text). The Table is an extension of that made by Laskowski, Jr.⁴⁸. For the avian ovomucoids⁴⁸ frequencies of appearance are shown in parentheses. Somewhat arbitrary homology relationships T = S; P = F; R = K, V = L = I were assumed. The cystines corresponding to Cys-71 and Cys-101 of SSI are connected in all the inhibitors. Solid outlines are for homologies involving SSI and dotted outlines are for those involving plasminostreptin only.

for the two inhibitors²². In the case of STI, which has no disulphide bridge close to the reactive site, there cannot be a similar secondary contact region, although some interactions other than those at the primary site are present⁶.

Relation to pancreatic secretory inhibitor

There is evidence for the close evolutionary relationship of pancreatic secretory (Kazal) trypsin inhibitors (PSTI)⁴⁵, acrosine inhibitors⁴⁶, canine submandibular inhibitors⁴⁷ and avian ovomucoids⁴⁸. Let us provisionally call this 'family' of trypsin inhibitors the PSTI family. Moreover, Ikenaka *et al.*¹⁴ discovered a homology between SSI and PSTI. To a recent exhaustive list of the PSTI family made by Laskowski⁴⁸, data for SSI¹⁴ and plasminostreptin³⁷ have been added (Table 2). In the reactive site (the primary contact region) the homology between the SSI sub-family (including plasminostreptin) and the PSTI family extends over 19 residues, ranging from Glu-67' to Val-85' of SSI. Another homologous region exists between Val-96' and Glu-102' of SSI¹⁴. In both the PSTI family and the SSI sub-family, the cystine corresponding to Cys-71' of SSI is connected to that corresponding to Cys-101' of SSI. Surprisingly, this second homologous region is exactly the same as the secondary contact region mentioned above. In SSI, the side chain of Glu-100' is exposed to the solvent, making no contact with either subtilisin or other parts of SSI. Thus the frequent replacement of this residue by a lysine having opposite charge is understandable. Asn-99', having no contact with subtilisin, seems to act as a spacer between the primary and secondary contact regions. The persistent conservation of this asparagine residue might reflect such a role. The replacement of this residue by histidine inactivates the inhibitor (Laskowski, personal communication).

Ser-98' makes contacts with Asn-155 and Glu-156. The replacement of this residue seems to be mostly restricted to the residues with stereochemically similar side chains. The same restriction seems to apply to Glu-102' the other residue making direct contact with subtilisin. The other highly conserved residues, Phe-97' and Val-96', make no contacts with subtilisin. However, Phe-97' seems to have the important role of fixing the secondary contact region to the rest of the molecule, with its benzene ring being tightly held by Met-103', His-106' and Arg-95'. Based on these findings it is very tempting to conclude that the inhibitors of the SSI sub-family and those of PSTI family are evolutionarily related despite their large phylogenetic distances. The inhibitor from leech⁴⁹ (see Table 2) supports this hypothesis. However, before it can be confirmed, many more

inhibitors from sources phylogenetically located between bacteria and mammals must be sequenced, and we plan a crystal structure analysis of a complex of PSTI with trypsin.

Origin of proteinase specificity

SSI strongly inhibits subtilisin BPN' but only weakly inhibits α -chymotrypsin and trypsin^{11,12}. BPTI and STI, on the other hand, strongly inhibit mammalian serine proteinases of the trypsin family but do not inhibit subtilisin BPN' (ref. 1). Using the same principle as that used for preparing Fig. 3, a hypothetical complex of subtilisin with BPTI or STI can be generated. In the case of BPTI, there are serious overlaps of atoms around Cys-38' of BPTI and around Gly-100 of subtilisin. Inspection of the model shows that these cannot be avoided by adjusting the relative orientation of the two molecules. The same situation exists for STI, where the overlaps are between atoms around His-71' of STI and Ala-99 of subtilisin. The reason that BPTI and STI do not inhibit subtilisin BPN' is simply due to the steric hindrance of atoms not belonging to the reactive site. In a complex of SSI with trypsin, on the other hand, there is no such obvious steric hindrance. The reason for weaker inhibition of trypsin¹¹ must lie in the reactive site itself. It should first be noted that P₁ of BPTI (Lys-15') and STI (Arg-63') form a strong ion pair with a carboxyl group (Asp-189) of trypsin^{3,6}. For SSI having Met-73' as P₁, the carboxyl group would constitute an isolated minus charge in the SSI · trypsin complex. In subtilisin BPN', there is no charged group in the S₁ crevice⁴³, and the fit of the methionyl side chain seems ideal. Plasminostreptin with lysine as P₁ moderately inhibits both trypsin and subtilisin BPN' ($K_1 \approx 10^{-8}$ M)³⁹. Apart from P₁, we note that P₂ is arginine in both BPTI and STI. The guanidium ion has no complementary charge in the corresponding crevice of trypsin and, in this sense, tyrosine (in SSI) or phenylalanine (in plasminostreptin) might be more favourable. The P₂ residue has a bulky group in both BPTI (Cys-14') and STI (Tyr-62'), but has only a moderately bulky group in SSI (Pro-72') and plasminostreptin (Thr-68'). Recently, Fujiwara *et al.*⁵⁰ estimated K_1 for the SSI · thiolsubtilisin complex to be 1.3×10^{-5} M at pH 7.0. The finding that the introduction of an atom with van der Waals radius larger only by 0.45 Å results in dramatic weakening of the association, suggests that the strong affinity between SSI and subtilisin BPN' is highly dependent on the precise geometrical fit between the two molecules.

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The *E. coli* gene encoding heat stable toxin is a bacterial transposon flanked by inverted repeats of IS1

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Restriction endonuclease subclones of the Escherichia coli gene encoding the heat stable (ST) toxin exhibit a stem and loop structure similar to those seen in many procaryotic transposons. An EcoRI DNA fragment encoding tetracycline (Tc) resistance but no transposition functions was spliced into the ST gene in one of these subclones. By monitoring Tc^r, we were able to show that the ST gene transposes. Restriction and DNA sequence data strongly suggest that the ST transposon, Tn1681, is flanked by inverted repeats of IS1.

ENTEROTOXIGENIC *Escherichia coli* have been implicated in diarrhoeal disease of pigs, cattle, sheep and man¹⁻⁵. Toxigenic strains of *E. coli* elaborate two types of toxins, one heat labile (LT) and the other heat stable (ST)^{6,7}. Like cholera toxin, LT stimulates adenyl cyclase; moreover it is immunologically cross reactive with cholera antitoxin^{8,9}. LT activity is assayed using either Chinese hamster ovary cells (CHO)⁸ or Y-1 adrenal mouse cells¹⁰. ST is small (less than 5,000 MW)^{11,12}, is nonimmunogenic and probably exerts its toxic effects via stimulation of guanylate cyclase^{13,14}. ST activity is assayed in suckling mice¹⁵. The genetic determinants for both toxins are plasmid borne and can occur together on the same plasmid or separately^{16,17}. We have reported the cloning of the gene which encodes the LT toxin as a DNA fragment of 3.0 kilobases¹⁸ and the gene which encodes the ST toxin as a fragment of 8.9 kilobases¹⁹. We report here the subcloning of the ST determinant into restriction fragments ranging in size from 3.4 to 0.4 kilobases. Detailed study of one of these subclones revealed that the ST gene resides on a transposon flanked by inverted repeats of IS1.

Subcloning the ST gene

The construction of a plasmid hybrid (ESF3001) consisting of an 8.9 kilobase *EcoRI* fragment encoding the ST toxin cloned into RSF2124 (ref. 19) has been described. Cleavage of this fragment with *Bam*HI results in two fragments of approximately 5.6 and 3.4 kilobases (Fig. 1a). These two *Bam*HI-*Eco*RI fragments were cloned separately in pBR322 which had been cleaved with *Bam*HI and *Eco*RI. Removal of the internal *Bam*HI-*Eco*RI fragment from pBR322 results in a tetracycline sensitive (Tc^s) phenotype since the *Bam*HI site is within the Tc resistance gene²⁰. Following transformation into *E. coli* RR1 (refs 20, 21), clones were selected for resistance to ampicillin (Ap) and then tested for sensitivity to Tc. The restriction pattern of plasmids from six clones exhibiting the Ap^r Tc^s phenotype were examined and found to contain either the 5.6 or 3.4 kilobase insert. Only the clones containing the 3.4 kilobase insert produced ST. One such clone was used for further study and was designated pMS2.

Digestion of the pMS2 insert with *Pst*I results in three fragments of 1.7, 1.4 and 0.3 kilobases (Fig. 1a). The central

1.7 kilobase *Pst*I fragment (Fig. 1b) was cloned in the *Pst*I site of pBR322. The single *Pst*I site within pBR322 occurs in a region encoding Ap resistance²⁰. Transformants were therefore selected for Tc^r and checked for sensitivity to Ap. Tc^r Ap^s clones contained the 1.7 kilobase insert and produced ST. One such clone was used for further study and was designated pMS3.

Characterisation of the ST clones

Examination of single-stranded DNA from the ESF3001 plasmid which contains the *Eco*RI ST⁺ fragment in the electron microscope²² reveals the presence of a prominent inverted repeat (double-stranded segment) flanking a shorter single-stranded region (Fig. 2a). Measurement of this double-stranded segment relative to native relaxed pSC101 DNA (9.4 kilobase) included in the same grid places the size of the inverted repeat at 760 base pairs. Examination of the *Bam*HI-*Eco*RI and the *Pst*I fragments shows that the inverted repeat is present in these inserts (Fig. 2b, c). The *Pst*I sites are apparently within the inverted repeat since the double-stranded portion of the single stranded *Pst*I fragment is shorter than the original length by approximately 180 base pairs.

Figure 1b shows a restriction map of the pMS3 insert. Since the *Pst*I fragment has an inverted repeat (IR), restriction sites within the IR would be symmetrically arranged and digestion with these restriction enzymes would yield two DNA fragments of identical molecular weight. Digestion of the *Pst*I fragment with *Alu*I yields fragments of 392 base pairs (two fragments), 102 base pairs (two fragments), 350 and 290 base pairs, when calibrated with *Hinc*II fragments of Φ X174 RFI DNA²³. The *Alu*I sites giving rise to the largest and the smallest fragments would therefore lie within the IR.

Many antibiotic resistance determinants are known to be transposable; they lie in discrete regions of DNA bounded by inverted repeated sequences (see ref. 24 for review). The cloned fragments of DNA containing the ST gene exhibit strikingly similar morphology. By analogy to known transposable genetic determinants, the ST gene would be expected to lie within the area comprising the loop in the stem and loop structure visualised in the electron microscope. Referring to the map (Fig. 1) the site would be located within the area delineated by the two middle *Alu*I fragments. These two *Alu*I fragments were purified and cloned separately in the *Hind*III site of pBR322. Since DNA cleaved with *Alu*I contains duplex or 'flush' ends, the *Alu*I fragments were ligated to synthetic decameric oligodeoxynucleotides, or 'linkers', encoding the restriction site for *Hind*III²⁵. Neither *Alu*I fragments thus cloned gave rise to an ST⁺ phenotype. In the same experiment we also cloned the 113 base pair *Alu*I fragment. These clones were also ST⁺.

In a separate experiment a partial *Alu*I digest of the *Pst*I fragment produced a double *Alu*I fragment consisting of the 350 and 290 base pair fragments. This *Alu*I double fragment was subsequently purified and cloned into pBR322 again using the *Hind*III linkers. Clones containing this 640 base pair fragment were ST⁺. It thus seems that the middle *Alu*I site is within an

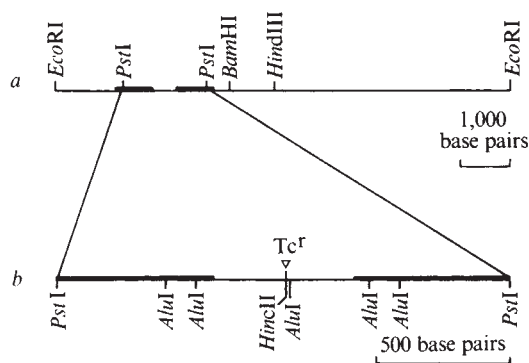


Fig. 1 Restriction maps of *a*, the 8.9 kilobase ESF3001 *EcoRI* insert and *b*, the 1.7 kilobase pMS3 *PstI* insert. Restriction enzyme analyses of DNA fragments were performed in conditions recommended by the suppliers. *AluI*, *BamHI* and *HincII* were obtained from New England Biolabs; *HindIII* from Boehringer-Mannheim. *PstI* and *EcoRI* were purified in the laboratory according to the procedure of Greene⁴². DNA fragments were electrophoresed in 0.8% agarose in Tris-borate buffer⁴¹, with *EcoRI* fragments of λ phage as molecular markers; or in 7% acrylamide in Tris-borate buffer, with *HincII* fragments of Φ X174 RFI DNA as markers²³. *AluI* sites were not mapped beyond the 1.7 kilobase *PstI* fragment.

area essential for ST expression. Plasmids containing this fragment are termed pMS6.

Restriction analysis of the pMS2 insert has already shown that there is a *HincII* site close to the centre of the fragment within 50 base pairs of the middle *AluI* site. To determine if this *HincII* site is also necessary for ST expression, we mutated the site by ligating into it the *HindIII* linkers. There are three *HincII* sites within pMS2, two of them within pBR322 (ref. 20). pMS2 plasmid DNA was digested with *HincII* in conditions which allowed on the average one cleavage per molecule, so that the majority of the DNA consists of unit-length linear molecules. Kinase-treated *HindIII* linkers were added at a molar ratio of decamer to plasmid of 25:1, and ligated with T4 polynucleotide ligase. The DNA was restricted with *HindIII* to generate cohesive termini and the preparation electrophoresed through an acrylamide gel. Unit-length linear DNA was extracted from the acrylamide, circularised with *E. coli* DNA ligase and used to transform *E. coli* RR1. Transformants were selected for Ap^r and the plasmid DNA analysed for the location of the new *HindIII* site. Plasmids containing the *HindIII* insertion at the location of the former *HincII* site had the ST⁻ phenotype. These plasmids were designated pMS2a.

Identification of the inverted repeats flanking the ST gene

Several lines of evidence strongly suggest that the inverted repeat flanking the ST gene sequence is IS1. The estimated size of the ST inverted repeat (760 base pairs) is similar to that given by Ohtsubo for IS1 (768 base pairs) (ref. 26). In addition, Ohtsubo reported that in IS1 there are two *AluI* sites within the region delineated by the *PstI* site and the right hand end of IS1. The sizes of these fragments are (*PstI*-*AluI*) 407 base pairs, (*AluI*-*AluI*) 113 base pairs, and (*AluI*-end of right end) 66 base pairs; these values agree well with those estimated for the corresponding fragments of the ST inverted repeat: (*PstI*-*AluI*) 392 base pairs, (*AluI*-*AluI*) 102 base pairs and (*AluI*-end of ST IR) 66 base pairs.

The nucleotide sequence of the pMS6 insert was determined using the Maxam and Gilbert procedure²⁷; the complete sequence will be reported elsewhere. The first and last 66 bases of the sequence are inversely repeated relative to each other. The top line of Fig. 3 shows the first 70 bases of the sequence. The first and last 66 bases comprise the end of the inverted repeat proximal to the loop as seen in the electron micrographs; comparison of the sequence with the last 66 bases of the right hand end of IS1 (ref. 27) (bottom line) show that the sequences are identical; thus, we conclude that the ST gene is flanked by inverted repeats of IS1.

Transposability of the ST gene

The lack of a direct method of selection for the ST gene has hampered efforts to test for its transposability. However, the restriction sites within an area essential for ST expression provided a means to test this possibility. Antibiotic resistance genes can be inserted into that region and the resistance marker used to monitor transposability and to measure the frequency of transposition. For these experiments, pMS2 was used because it contains the entire inverted repeat. Octameric linkers containing the recognition sequence for *EcoRI* was ligated into the *HincII* site of the *BamHI*-*EcoRI* insert as described above. A 2.4 kilobase *EcoRI* fragment encoding resistance to Tc but specifying no transposition functions was ligated into the newly generated *EcoRI* site. This fragment was originally cloned out of pBR322 using *EcoRI* linkers²⁸. As the cloning vehicle retains a portion of its own tetracycline resistance genes, the resultant hybrid was transformed into the *recA* strain MV12(RSF2001) to preclude recombination with the newly introduced Tc^r genes. RSF2001 is a kanamycin resistant derivative of F (ref. 29). Transformants were selected for resistance to Tc and plasmid DNA from these clones were examined for the presence of the *EcoRI*-Tc^r fragment. One clone containing this fragment was used for the transposition studies and is designated pMS2b.

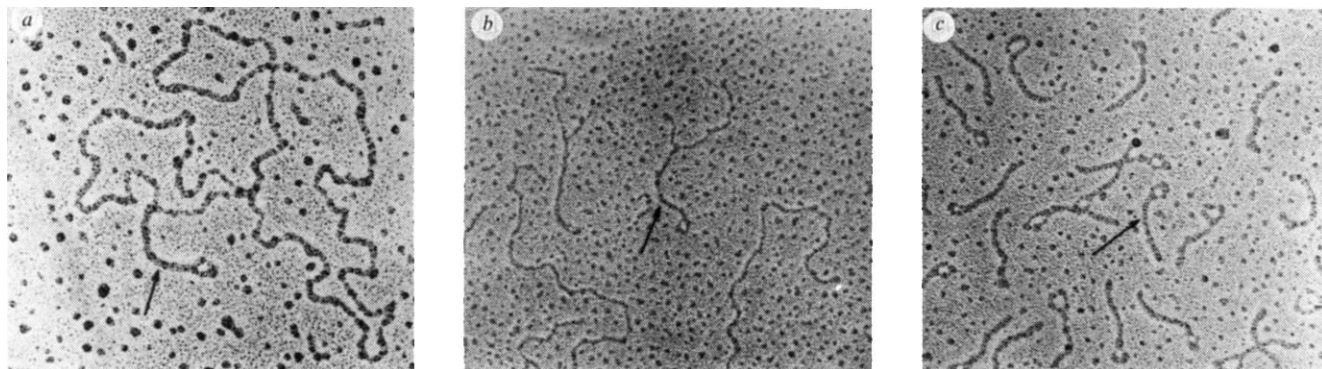


Fig. 2 Electron micrographs of single stranded DNA of *a*, ESF3001 plasmid; *b*, the 3.4 kilobase pMS2 *BamHI*-*EcoRI* fragment; *c*, the 1.4 kilobase PMS3 *PstI* fragment. Preparations of single stranded DNA fragments for microscopy were obtained by the procedure of Davis²², and were examined using a JEOL 100B or a Philips 301. Micrographs in (*b*) and (*c*) were taken at the same (28,000) magnification.

ST IR CTGTCATGACAAAGTCATCGGGCATTAATCTGAACATAAAACACTATCAATAAGTTGGAGTCATTACATTA....
 IS1 ATCGGTGGAGCTGCATGACAAAGTCATCGGGCATTAATCTGAACATAAAACACTATCAATAAGTTGGAGTCATTAC

Fig. 3 Nucleotide sequence of (top line) the first 70 bases of the pMS6 insert encoding ST, and (bottom line) the last 76 bases of the right hand end of IS1 (as published by Ohtsubo²⁶). The nucleotide sequence of the pMS6 insert was determined as described by Maxam and Gilbert²⁷. DNA fragments ³²P-labelled at one 5' terminus were obtained either by cleavage of the labelled fragment with a restriction enzyme or by strand separation²⁷.

A method for the detection of transposition of an antibiotic resistance gene from a ColE1 type plasmid has been described previously²⁹. ColE1 has a strict dependence on the *polI* function of *E. coli*³⁰ while F-type plasmids do not. The strain MV12(RSF2001, pMS2b) was mated in 2.0 ml L broth with the nalidixic acid resistant (Nx^r) *polI* strain SF800 (ref. 29) at a donor to recipient ratio of 1:3. After 1 h of gentle shaking at 37 °C cells were plated on fresh MacConkey agar containing 20 µg ml⁻¹ Nx, 5 µg ml⁻¹ Tc. Since pMS2b is a ColE1 derivative, it cannot be maintained in SF800; Tc^r transconjugants arise only on transposition of the *EcoRI*-Tc^r fragment from pMS2b to the plasmid RSF2001 and the subsequent conjugal transfer of RSF2001Tc^r to SF800. Nx^rTc^r clones were observed in three separate mating experiments. Six of these clones were isolated and all were able to retransfer Tc^r and Kan^r together in subsequent 1-h matings with HB101 or RR1 as recipients. The frequency of transposition of the *EcoRI*-Tc^r fragment by the ST transposon, expressed relative to the frequency of RSF2001 transfer to SF800, is $\sim 4 \times 10^{-6}$. In the same experiments, MV12(Esf2001,pBR322) yielded Nx^rTc^r transconjugants in frequencies of $< 0.01 \times 10^{-6}$ when crossed with SF800.

We examined 20 of these Tc^rKan^r exconjugant clones from the MV12(RSF2001,pMS2b) × W3110 matings and found them to be also Ap^r and colicin immune indicating that the RSF2001 recipient plasmid contains the ColE1 portion of the pMS2b genome in addition to the ST gene. This event is IS1 mediated and is *recA* independent. These observations are analogous to those of Jeffrey Miller (personal communication) on Tn9, a transposon flanked by direct repeats of IS1 (ref. 31). Using the same detection system he observes that transposition of Tn9 from a ColE1 derivative onto F results in plasmid cointegrates in which two complete copies of Tn9 have inserted into F as direct repeats flanking ColE1. Our preliminary results suggest the same sequence organisation in the recipient plasmid. In view of the observation that the transconjugants are in fact plasmid cointegrates it can be argued that the ST gene is not a true transposon. However, in *rec⁺* conditions the flanking direct repeats of the ST sequences recombine, leaving only a single copy of the ST transposon on RSF2001. Thus the end product is identical to a transposition event and it would be difficult to argue that transposition does not normally occur with such a transitory intermediate. In fact, transposition of certain mutants of Tn3 onto RSF2001 results in cointegrate plasmids with an identical sequence arrangement (Tn3-ColE1-Tn3) (ref. 32). Perhaps we are observing in these Tn3 mutants what would normally be an intermediate in the transposition process.

Discussion

Many plasmid mediated bacterial genetic determinants are known to be on transposons. They were first characterised in genes specifying antibiotic resistance in *E. coli* and *Salmonella* (see ref. 24 for review). More recently, transposons have been described in *Pseudomonas* specifying enzymes involved in resistance to mercuric ions³³ and in the degradation of toluenes and xylenes³⁴, and in *Yersinia* specifying enzymes for the utilisation of lactose³⁵. To our knowledge the ST gene is the first pathogenic determinant shown to be on a transposon.

Most transposons are flanked by repeated sequences whose identities have yet to be established. Tn10 is reported to be flanked by inverted repeats of IS3 (ref. 31) while in the case of Tn9 the chloramphenicol resistance (Cm^r) genes are flanked by

direct repeats of IS1 (ref. 36). Direct repeats of IS1 have also been found on several R factors of the *fi⁺* class bracketing the resistance determinants (see ref. 37 for review). The ST transposon is the first instance in which the ends are flanked by inverted repeats of IS1. That IS1 can occur in both orientations suggests that the directionality of IS1 is not an important factor in transposition. On the other hand, Ohtsubo has reported the presence of short terminal inverted repeats within the IS1 sequence itself; thus regardless of the orientation of the two IS1, the intervening genes still would be flanked by inverted repeats. And it is these internal inverted repeats which may play an essential role in transposition.

Transposable elements known to date confer easily detectable phenotypes. This, in fact, is how their transposability was first observed. Many other genetic determinants are not as amenable to such analysis because, like ST, their detection is difficult. Splicing an antibiotic resistance marker into such a gene to monitor transposition is a useful general strategy in such cases. The transposability of the ST gene accounts for the heterogeneity of the ST plasmids, which differ in their molecular size, %G+C content and incompatibility grouping³⁸. A *PsII* fragment of similar molecular weight encoding the ST toxin has been cloned from an *E. coli* enteropathogenic for pigs (N. Harford, personal communication). It would not be surprising then if the ST toxins elaborated by *E. coli* strains are closely related if not identical proteins.

Simple acquisition of an ST plasmid does not render an *E. coli* pathogenic. Besides producing ST, a strain must also elaborate an adherence or colonisation factor (CF) before it can become fully pathogenic^{1,2,39,40}. Such factors are plasmid encoded and are cell and animal specific. The colonisation factor is expressed as filamentous cell surface proteins which enable the organism to adhere to the mucosa of the upper bowels of the animals, an area normally devoid of such organisms. In addition, diarrhoeagenic *E. coli* isolated from pigs elaborate a CF different from those isolated from calves or man. In the course of association between an ST and a CF plasmid within a pathogenic *E. coli* one would expect the eventual acquisition of the ST gene by a CF plasmid. Indeed such a case has been found. A plasmid encoding the human colonisation factor has been shown to harbour the ST gene as well (P. Shipley, personal communication).

Other pathogenic determinants such as the heat labile (LT) toxin and the K antigens may also reside on transposons. Silva *et al.* have reported the presence of inverted repeats bounding an area known to contain genes for both the ST and the LT in a plasmid isolate of an enteropathogenic *E. coli*⁴¹. Transposition has proved to be an important mechanism in the dissemination of antibiotic resistance; it may ultimately prove to be as common a mechanism as conjugation and phage conversion by which pathogenic determinants are acquired by bacterial genomes.

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letters

Death throes of massive stars

MASSIVE stars undergo violent deaths, forming the type II supernovae believed to result from gravitational collapse of the stellar cores. Such an event may be recognised by the optical outburst which occurs when the thermal and kinetic energy generated by the collapse propagates to the stellar photosphere. This occurs ~5–20 d after the collapse¹. However, the optical data are incomplete in the sense that supernovae are invariably discovered at or shortly after peak optical intensity so only the decline from maximum brightness has been recorded in any detail. In addition, various theoretical models suggest that the optical radiation will be preceded by bursts of other forms of energy (neutrinos, gravitational waves, γ and X rays). Detection of these bursts, as well as of the early part of the optical light curve, would be greatly facilitated if the occurrence of a supernova could be predicted on the basis of the pre-supernova behaviour of the star. We show here that neutrino emission in the final stages of nuclear burning (as originally proposed by Morrison²) could, in principle, provide such a warning, though the current practical limitations are severe.

Our results are based on a study of the final stages of evolution of a $15M_{\odot}$ star (details will be published elsewhere). The evolution through hydrogen, helium, and carbon burning was computed with a hydrostatic stellar evolution code³. The subsequent stages (neon, oxygen, and silicon burning) were followed with an implicit hydrodynamic code⁴. The nuclear reaction network included the species ^1H , ^4He , ^{12}C , ^{16}O , ^{20}Ne , ^{24}Mg , ^{28}Si , ^{32}S , ^{36}Ar , and ^{56}Fe . Neutrino emission was computed

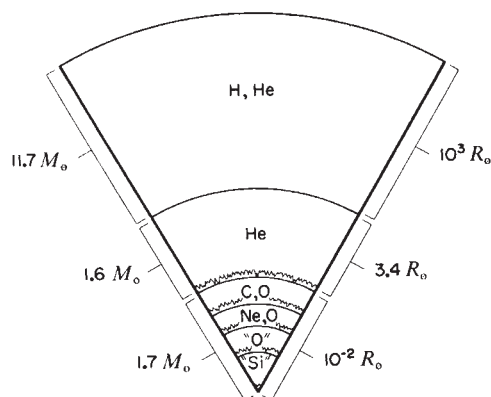


Fig. 1 Cross-section (not to scale) of the star at the beginning of the silicon-burning phase. Jagged lines indicate active burning regions. The composition of the O and Si zones consist of a number of species; 95% of the mass in the inner ($1.7M_{\odot}$) region is contained in the Si zone.

according to the rates of Beaudet *et al.*⁵ which include the pair-, photo-, and plasma-neutrino processes. The Weinberg-Salam model for weak interactions suggests that these rates should be revised upwards⁶, but experimental evidence⁷ indicates that the correction is less than a factor of 2. All of these processes produce equal numbers of (electron) neutrinos and antineutrinos.

The structure of the star at the start of the period of intense neutrino emission is shown in Fig. 1. This represents the beginning of the core-silicon-burning phase. The results of the calculations for the subsequent evolution are shown in Fig. 2, in which nuclear burning and neutrino energy emission rates are plotted against time. Average neutrino energies at representative times are also indicated. As the dominant neutrino emission process is the destruction of electron-positron pairs, the neutrino energies include the 0.511 MeV rest-mass energy of the annihilated particles. The first (broad) peak in neutrino emission represents core silicon burning, which occurs several months before collapse, and subsequent (sharp) peaks are due to increasingly violent shell flashes. The last major burst corresponds to a silicon flash ~4 d before collapse. We should emphasise that, although the nuclear energy generation rate varies by 7 orders of magnitude during this period, no detectable variations in the surface photon luminosity occur. To an optical observer, the star appears as a perfectly normal red supergiant and gives no warning of the impending explosion.

To examine the detectability of the predicted fluxes, the background levels over which the stellar neutrinos (or antineutrinos) must dominate should be considered. For this purpose we choose the last peak, at which time the neutrino (plus antineutrino) luminosity is $6 \times 10^{11}L_{\odot}$ and the average energy per particle is 0.83 MeV. For the neutrinos, the main background is the Sun. Optimal discrimination against solar neutrinos is afforded by a detector which is insensitive to the copious p-p neutrinos, such as the ^{37}Cl experiment of Davis⁸. The condition that the capture rate for stellar neutrinos be greater than for solar neutrinos is

$$\sigma_s F_{\nu s} > \Sigma \sigma_{\odot} F_{\nu \odot} = 1.6 \times 10^{-36} \text{ s}^{-1} \quad (1)$$

where we have used the measured value for the solar neutrino capture rate⁹. With $\sigma_s = 2.4 \times 10^{-46} \text{ cm}^2$, we find that the pre-supernova object would have to be within 34 pc to be detected. Obviously this is unacceptable. Note that Bahcall¹⁰ has estimated that the neutrino burst due to core collapse would be detectable at a distance of 4.5 kpc. However, radiochemical detectors are generally not very suitable for supernova prediction as they involve long integration times and give no directional information.

The situation is more favourable for the antineutrinos, where the solar background is negligible and the main background is

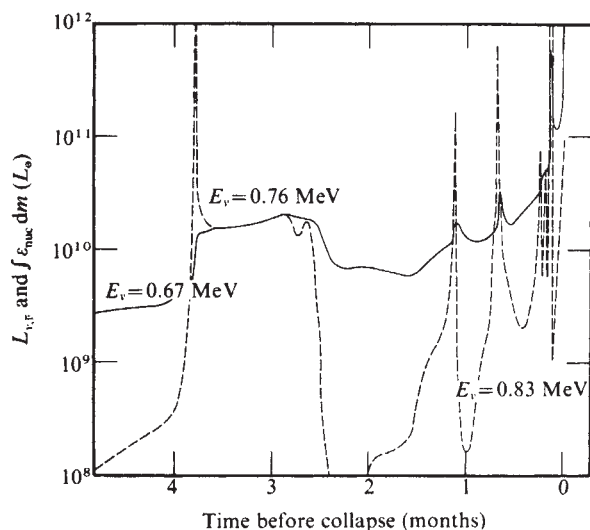


Fig. 2 Total energy emission due to neutrino pairs (solid line) and energy generation by nuclear reactions (dashed line) as functions of time. Average neutrino energies are indicated at various points. The $E_{\nu} = 0.83$ MeV notation refers specifically to the last silicon flash.

due to radioactive decays in the Earth's crust. The flux of terrestrial antineutrinos is not well known, but it is estimated to be $F_{\nu\bar{\nu}} \sim 3.5 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$, if we eliminate the decays with peak energies below 0.5 MeV¹¹. As the remaining decays yield energies comparable to the expected stellar antineutrino energies, we can directly compare fluxes, without referring to cross-sections for a particular detector. The condition $F_{\nu\bar{\nu}} > F_{\nu\bar{\nu}}$ now leads to a distance limit of 1.5 kpc. With an expected event rate of ~ 0.1 supernova yr^{-1} in the entire Galaxy, this is not very encouraging. If we wish to monitor the entire Galaxy, a space experiment would be necessary. For a satellite in geosynchronous orbit, the terrestrial antineutrino background would be negligible, although the technical difficulties involved in designing a suitably sensitive detector should not be underestimated.

Antineutrinos emitted by massive stars in the final stages of nuclear burning could then be used to predict supernovae, though major improvements in detection techniques would be required. Note that the results presented above apply to a particular stellar mass ($15 M_{\odot}$) and that other masses might give substantially higher fluxes.

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An ultraviolet spectrum of the high redshift quasar Q2204–408

AN ultraviolet spectrum of the radio-quiet quasar Q2204–408, redshift $z = 3.18$ (ref. 1), detected with the International Ultraviolet Explorer (IUE) is reported here. The object was selected for observation because of its high Lyman- α flux and the absence of a cut-off at the Lyman limit. The spectrum was obtained on 9 August 1978 with the short wavelength spectrograph which covers the range 1,150–1,950 Å (ref. 2). A finder field for Q2204–408 has been given by Osmer and Smith¹ and as the quasar is too faint ($m_v = 17.5$) to be detected by the fine error sensor of IUE, a blind off-set technique was used to image the quasar in the large (10×20 arc s) aperture of the short wavelength spectrograph. A very faint continuous spectrum of average signal ~ 5 DN above a background of ~ 60 DN was detected after an exposure of 350 min. (DN stands for data number and 256 DN gives full scale.) An emission feature is also probably present at 1,270 Å (304 Å in the rest frame of the source).

A computer program was developed to track the spectrum in both the raw and the photometrically corrected images. The data, in a strip 18 pixels wide and centred on the spectrum, were extracted without rebinning and only wavelengths longer than the night-sky Lyman α line have been considered. The data in the photometrically corrected image were compared with those in the raw image, and a visual examination made of the photo-write of the raw image so as to identify and remove signals caused by cosmic ray events and other noise spikes. The data in the central 6-pixel wide strip were combined and a background correction made by subtracting the smoothed signal on either side, thus giving the net spectrum. The signals were converted to absolute fluxes with the most recent³ absolute efficiency of the short wavelength spectrograph. A scaling factor was used to convert the flux value obtained with the data extracting procedure used here to that which would be obtained with the extraction procedure used to derive the absolute efficiency of the spectrograph. This was determined to be ~ 1.2 by reducing the spectrum of the quasar MKN205 (also observed by us) with the two extracting procedures, and comparing the outputs.

The spectrum of Q2204–408 was averaged in 100 Å bands and the resulting fluxes and central wavelengths are given in Table 1. The r.m.s. errors are also given and the increase at the longer wavelengths is due to a high local background that was accumulated during the long exposure. The data between 1,400 and 1,500 Å have not been used, as they lie in a sensitivity minimum of the instrument (caused by the photocathode in the camera) where the signal becomes too low to be significant. Because of the large errors, the UV data have been combined to give the weighted mean listed in Table 1, where the error has been increased to allow for the uncertainty of $\sim 10\%$ in the absolute calibration³. The UV flux can now be compared with the visible flux measurement of Osmer and Smith¹ also given in the Table.

Table 1 Fluxes and central wavelengths for Q2204–408

Wavelength (Å) Rest frame of observer	Flux ($10^{-16} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Å}^{-1}$)	Wavelength (Å) Rest frame of source
1,350	4.2 ± 1.0	323
1,650	10.4 ± 1.4	395
1,750	9.9 ± 4.9	419
1,850	5.5 ± 3.4	442
1,480*	6.3 ± 1.0	354
6,165†	2.9	1,475

*UV weighted mean.

†From Osmer and Smith¹.

As usual, we assume a continuum flux distribution in frequency terms of the form $f(\nu)\alpha\nu^{-\alpha}$ and from the UV and visible data derive a value for the spectral index $\alpha = 1.5 \pm 0.3$. This is in the range found for quasars from optical and radio data and shows that the non-thermal continuum extends to wavelengths as short as $\sim 300 \text{ \AA}$ in the rest frame of the source. It also confirms the great transparency of the intergalactic medium which has been well established by several workers (see, for example, refs 4, 5). A difference here is that the observed flux has been subjected to the full absorption characteristics of neutral hydrogen, that is, in the Lyman continuum and Lyman series, and this occurs in a local epoch ($z < 1$). Additionally, the flux will suffer absorption by any intergalactic neutral helium in the early epoch $z \sim 2-3$.

The extracted spectrum shows an emission feature near $1,270 \text{ \AA}$ which is probably real. Its wavelength is uncertain because the only internal standard is provided by the night-sky Lyman α line whose signal is saturated. However, the wavelength is clearly close to $304 \text{ \AA}$ in the rest frame of the quasar and the feature is likely to be the redshifted HeII resonance line. Its total intensity is estimated as $\sim 2 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1}$ which can be compared with the intensity of (Lyman $\alpha + \text{Nv}$) $= 11 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1}$ given by Osmer and Smith¹. Further observations are being made which should confirm the reality of the feature.

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The August 1972 solar proton event and the atmospheric ozone layer

OXIDES of nitrogen are generally accepted to be important for regulating the atmospheric ozone content through catalytic reactions first described by Crutzen¹ and Johnston². In the stratosphere, nitric oxide is produced naturally by the reaction of nitrous oxide, emanating from the biosphere, with excited oxygen atoms generated by ozone photolysis at wavelengths shorter than about 310 nm . As Crutzen *et al.*³ have pointed out, large amounts of nitric oxide may also be produced by high-energy solar protons, mainly at geomagnetic latitudes greater than 60° , and altitudes above about 35 km . The ozone measurements reported here made during an intense solar proton event in August 1972 suggest that NO production rates due to ionising particles are higher than those calculated by Crutzen *et al.*

This process of NO production is likely to occur in several steps: nitrogen molecules are ionised and dissociated by secondary electrons with energies in the range of tens to hundreds of electron volts. The subsequent reaction $\text{N} + \text{O}_2 \rightarrow \text{NO} + \text{O}$ is slow

and temperature dependent for ground N (^4S) atoms, but not for electronically excited N (^2D or ^2P) atoms. As the electronic states of the N atoms were not known, Crutzen *et al.*, in their computation of NO production rates for three major solar proton events, considered two extreme situations with either complete or no production of N atoms in the excited states.

Recent rocket measurements of mesospheric and thermospheric nitric oxide concentrations revealed strong enhancements during auroral particle precipitation events (ref. 4 and F. Arnold, personal communication). From these measurements, and simultaneous measurements of ionisation rates, it is inferred that the NO-production rate is about 2–2.5 times the ionisation rate, while Crutzen *et al.* base their estimates on 1.5 mol per ion pair, as given by Winters⁵. These values imply that most of the N-atoms resulting from electron impact dissociation of N_2 are probably in an electronically excited state⁴.

On 4 August 1972 a solar proton event occurred that was the most intense of any recorded within the past 25 yr. Ozone measurements made with the BUV experiment aboard the Nimbus 4 satellite during that period were the first ever to show unambiguously a decrease in stratospheric ozone due to a solar proton event⁶. Figure 1 shows total ozone amounts above the 4 mbar level, for three latitude belts, as published by Heath *et al.*⁶. Zonally averaged daily values are plotted with the respective error bars for the months of July and August 1972. In the polar region, between 75° and 80° N , the event produced an abrupt decrease in ozone above 4 mbar, of about 0.002 atm cm or 16%, which apparently persisted throughout the month of August. At latitudes between 55 and 65° N a very sharp drop was noticed 2 d after the event followed by a slow decrease of 5% within 8 d, and a gradual 'recovery' thereafter. Heath *et al.* interpreted this behaviour as being due to the advection of modified air from the source region, which is symmetric in geomagnetic coordinates by geographically orientated zonal motions, with the succeeding 'recovery' being similar to the seasonal changes found in other years in this latitude range. The

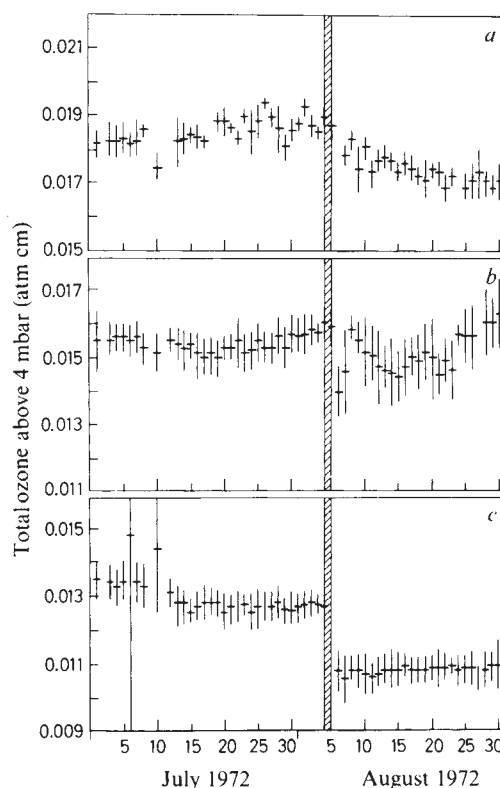


Fig. 1 Zonally averaged total O_3 , above the 4-mbar pressure surface for equatorial (a), middle (b), and high latitudes (c) during July and August 1972. The solar proton event occurred on 4 August. After Heath *et al.*⁶.

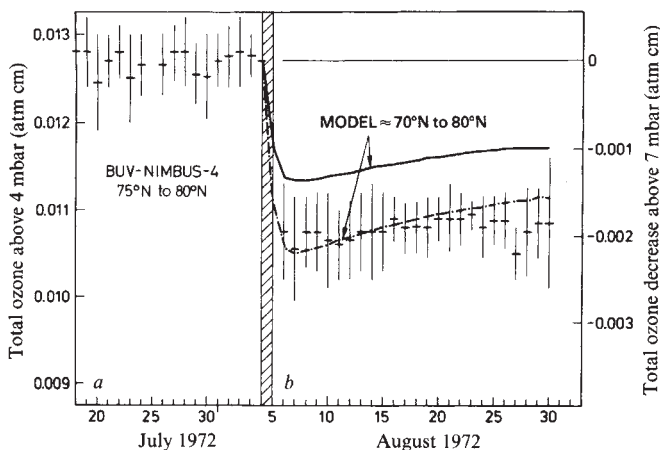


Fig. 2 Comparison of BUJ-NIMBUS-4 ozone data (a) with model prediction for high latitudes (b). The model results are plotted as relative depletions with respect to the 'undisturbed' level on 4 August, before the event occurred. The solid line bases on NO production rates as given by Crutzen *et al.*³. The dashed line includes Arnold's modification⁴.

ozone measured in the tropics are, although different from those observed in earlier years, not related uniquely to the proton event. According to Heath *et al.* the slow ozone increase during July and the steeper decline in August are likely to be partly due to an abnormal temperature decrease in July associated with a Southern Hemisphere stratospheric warming, followed by a recovery in August.

As Crutzen *et al.*³ pointed out, studies of the ozone distribution in the stratosphere in periods following certain proton events, may be important for validating numerical models of photochemistry and transport processes in the stratosphere. We have performed such modelling experiments using the Oxford zonally averaged, two-dimensional model, described in detail by Harwood and Pyle⁷. This time-dependent model extends from the South Pole to the North Pole, with 19 latitude belts of slightly less than 10° width, and from the Earth's surface to about 80 km altitude, with a height increment of about 3.5 km (0.5 pressure scale heights). Eddy fluxes of heat and matter are treated using large-scale diffusion coefficients, while the momentum fluxes are specified from temperature measurements made mainly by the selective chopper radiometer on the Nimbus 5 spacecraft. Radiative processes, such as the absorption of solar radiation by ozone and the radiative cooling due to the CO₂ and O₃ emissions, are included in the model. The photochemical scheme, which was specified in the lowest 17 layers corresponding to the lowest 60 km of the atmosphere, comprises the most important photochemical reactions determining the concentrations of oxygen, nitrogen, and hydrogen compounds. Continuity equations are written for the following species or groups of species: O, O(¹D), O₃; N, NO, NO₂; HNO₃; H₂O₂; H, OH, HO₂ (ref. 8). For the latter group the photochemical time scales are short and dynamical processes are ignored.

The solar proton simulation experiments were performed after the model had reached a quasi steady state thus ensuring that all trace gas variations were merely due to seasonal changes. On 4 August, when the event occurred, a sudden injection of NO was introduced in the model. In a first experiment we used NO production rates for the August 1972 event as calculated by Crutzen *et al.*³ for excited N atoms. (In fact, the differences of NO production rates between ground-state and excited N atoms only become important for altitudes above 50 km, whereas the highest ozone depletion rates through this effect are found below that altitude. Additional test runs showed that the ozone depletions for both cases differ by 7%.) A second experiment was run with the same set of NO injection rates, but multiplied by a factor of 1.7 as suggested by Arnold's findings. For both

cases the solar proton-produced NO was introduced at geographical latitudes greater than 60°. It is inherent in our zonally averaged approach that geomagnetic and geographic latitudes are assumed to be coincident. The results of these experiments are given in Figs 2 and 3 for polar and mean latitudes, respectively. For comparison with the BUJ data as taken from Fig. 1 (scale on a) the model results are plotted as relative depletions with respect to the undisturbed conditions immediately before the proton event occurred (scale on b). Total ozone was integrated above the 6.8 mbar level of the model which yielded the best match with the total ozone amounts indicated, obtained above 4 mbar by the BUJ experiment.

For the polar latitudes (Fig. 2) the model results fit the observations reasonably well when the NO production data by Crutzen *et al.*³ are increased by 1.7 as discussed earlier (dashed line). Without this modification, as shown by the solid line, the predicted ozone drop due to the proton event is too small. The same holds for the model results for the 55 to 65° latitude belt shown in Fig. 3. As this latitude region is covered by two adjacent latitude belts in the model, that is ~50 to 60° and ~60 to 70°, predictions of ozone depletion for both are plotted separately. Superimposing the two dashed lines, corresponding to modified NO production rates, there is, within the accuracy indicated by the error bars, good agreement with the BUJ observations, before the 'recovery phase' which starts after about 20 August. If the interpretation offered by Heath *et al.* for the mid-latitude changes is correct, we should not expect a zonally averaged model to reproduce all the details of the effect. Furthermore, one should bear in mind that the ozone shows natural variations illustrated best by those observed in the tropical region (Fig. 1a) where the solar proton event did not cause any measurable effect.

We have investigated the changes in the ozone budget in the model following the event. Thus we have separated the net rate of change of ozone into contributions due to the photochemical and the mean and eddy transport terms. In high latitudes, the increased photochemical destruction during the event is counteracted to some extent by the transport. For instance, between about 60° and 70°N, where the perturbations to the ozone concentration are largest, the eddy terms tend to increase the ozone while the mean motion terms, which on average remove ozone from this region, are reduced in magnitude following the event.

It is interesting that the model overestimates the ozone recovery after about 23 August, at the highest latitudes considered, but underestimates it in middle latitudes. It is possible that the meteorological conditions prevailing at this particular

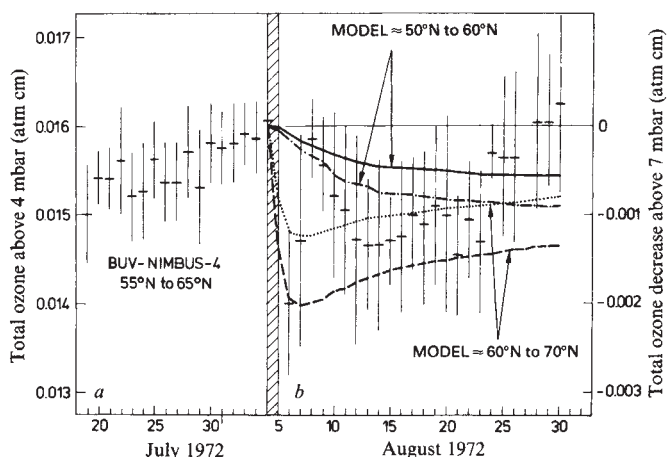


Fig. 3 Comparison of BUJ-NIMBUS-4 ozone data (a) with model prediction for midlatitudes (b). The 55 to 65°N latitude range of the BUJ data is somewhat between the latitude belts 50 to 60°N and 60 to 70°N of the model. The solid line and the pointed line correspond to NO production rates given by Crutzen *et al.*³ for the two latitude belts, respectively. The dashed lines include Arnold's modification⁴.

time were such that there was a transport of ozone from high to middle latitudes, larger than calculated in the model. Clearly a model using monthly diffusion coefficients and momentum fluxes cannot be expected to reproduce dynamical changes on a synoptic time-scale. For this reason we do not discuss here our results for the Southern Hemisphere, where the calculated ozone changes were smaller, because the circulation, and thus the ozone distribution, would have been dominated by the aforementioned stratospheric warming. The model did predict temperature and circulation changes following the event but these were small, with temperature reductions not greater than 1% in the region of the greatest ozone changes. Using data from the Nimbus 4 selective chopper radiometer, the temperature sounding instrument built by the Department of Atmospheric Physics at the University of Oxford, we have looked for changes in temperature following the event. The top channel of the instrument provides information on the temperature averaged over about a 10 km thick slab in the upper stratosphere. The radiance data from this channel showed no significant changes associated with the event. It would appear that the feedback between the ozone concentration and temperature was not particularly important in this case. Indeed, our preliminary runs in which this feedback was suppressed, produced substantially the same results.

The results suggest that the NO production rates due to ionising particles are possibly higher than those originally calculated by Crutzen *et al.*³ although the theoretical study of Porter, Jackman and Green⁹ indicates even smaller rates. This work stresses the importance of the study of such natural phenomena for our understanding of atmospheric photochemistry and dynamics. It would be a major advance if, during one of the next major solar proton events, other related minor constituents could be measured. In this respect, satellite experiments such as those on Nimbus G, gain additional importance.

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Energetic protons near the plasma sheet boundary

It has long been evident that energetic particles are produced in the Earth's magnetotail in association with magnetospheric substorms^{1–6}. These particles may be important in producing ionospheric effects such as auroral zone absorption and they also lead to the injection of energetic particles into the interplanetary medium. We provide here an interpretation for the behaviour of a class of very intense, short-lived bursts of protons and other

ions which appear at the edge of the plasma sheet during the expansion phase of substorms.

The characteristics of impulsive proton bursts in the magnetotail were first described by Sarris *et al.*⁷ using observations of energetic proton and electron fluxes with good time resolution (~ 10 s) made at a geocentric distance of $\sim 35 R_E$ by experiments on the spacecraft IMP 7 and 8. The bursts are observed only on the dusk side of the magnetotail, have durations of 10–30 s at a given energy (perhaps depending on the energy resolution of the detectors) and are not detected beyond ~ 1.8 MeV in energy. The differential proton intensities at ~ 300 keV can be as large as $10^5 \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ MeV}^{-1}$ and they are highly collimated along the magnetic field direction with anisotropies as large as $10^5:1$ directed towards the tail. The most important features of the bursts is that they exhibit an inverse velocity dispersion. That is, the bursts are observed first at low energies and at higher energies some 10–20 s later, frequently when the low energy proton burst has already subsided. In general, energetic electron bursts are not observed in association with these impulsive proton bursts.

At least 12 of these bursts have been found and they have been shown by Roelof *et al.*⁸ to be directly associated with the expansion phases of large geomagnetic substorms. An examination of the University of Iowa LEPEDA spectrograms (L. A. Frank and K. L. Ackerson, personal communication) reveals that the bursts occur near the outer boundary of the plasma sheet during times when the plasma sheet disappears from the spacecraft (that is, during 'thinning'). Measurements of the magnetic field on the same spacecraft indicate that, in the case described here, the burst coincided with a transient perturbation of the magnetic field in both magnitude and direction (N. F. Ness and R. Lepping, personal communication). Observations of multiply-charged species indicate that in this event these particles behave in a similar fashion to that described above for protons (G. C. Gloeckler, personal communication).

Figure 1 shows the responses of the P1 (0.29–0.50 MeV), P2 (0.50–0.97 MeV) and P3 (0.97–1.85 MeV) channels of the

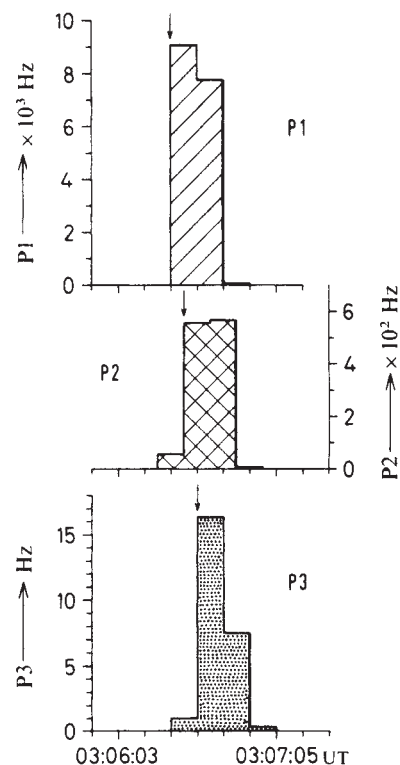


Fig. 1 Count rates of the P1 (0.29–0.50 MeV), P2 (0.50–0.97 MeV), and P3 (0.97–1.85 MeV) channels with a 10-s time resolution during the impulsive proton burst described in the text. (JHU-APL IMP 7 experiment, 27 March 1973.)

JHU-APL IMP 7 experiment during an impulsive proton burst which took place on 27 March 1973 at about 03.06.30 UT. The spacecraft was situated in the dusk magnetotail with solar magnetospheric coordinates $x = -31.5 R_e$, $y = 16.6 R_e$, $z = -5.0 R_e$. The LEPEDEA spectrograms show that the burst was associated, to within the accuracy allowed by the observations, with a plasma sheet boundary crossing in which the plasma sheet disappeared from the position of the spacecraft. The duration of the main pulse in each energy channel was ~ 20 s. The University of Maryland experiment on the same spacecraft, using a detector with a smaller energy window (125–160 keV per charge, $z \geq 1$), found the pulse width to be noticeably smaller, namely ≤ 5 s (G. C. Gloeckler, personal communication). It is clear from Fig. 1 that the low energy (P1) burst was observed first and that it was followed ~ 10 s later by the higher energy (P3) burst. The arrows on the figure mark edges of the burst where the proton intensities increase sharply by more than an order of magnitude.

There was no electron burst associated with the proton burst, although weak fluxes of electrons were present before the burst. It is possible however, that a very short-lived ($\ll 1$ s) electron burst could have occurred and escaped detection as a consequence of the ~ 1.3 s spin period of the spacecraft. A telescope responding to α -particles in the energy range $0.64 \leq E \leq 1.17$ MeV per nucleus indicated that no α -particles were present in this energy range. The University of Maryland experiment detected multiply-charged ions ($z \geq 2$) with an intensity about 10% of that of all particles with $z \geq 1$ and apparently having a rather sharp energy cut-off in the range 800–1,200 keV. We have estimated the possible contribution of heavy ions to the P1, P2 and P3 counting rates using the observations of magnetospheric ion bursts reported by Fan *et al.*⁹ as a basis, and find that such ions are likely to make a significant but small contribution to the counting rates. However, on the basis of the measurements presently available we cannot rule out the possibility that helium and oxygen ions (perhaps singly-ionised) make a substantial contribution to the counting rates which we have attributed to protons.

The properties of the proton bursts described above are consistent with the presence of a field-aligned sheet-like distribution of energetic protons which moves rapidly past the point of observation. The particles appear to have originated at a point on the sheet lying between the position of the spacecraft and the Earth and the magnetic field lines must be open so that the particles can escape freely. There is no evidence for any north-south difference in the occurrence of the bursts so that it can be expected that similar sheets exist simultaneously on both sides of the plasma sheet. The two sheets may therefore meet at a line where the particles originate, and this may be associated with magnetic field reconnection taking place at a neutral line which is (temporarily) situated between the spacecraft and the Earth¹⁰. In the following we examine the whole phenomenon from this point of view.

The situation must be such that the magnetic field lines which are reconnected at the neutral line are open, 'lobe' field lines (that is, with one end connected to interplanetary space) and with the plasma concerned being part of the very low density population observed in the lobes of the magnetotail¹¹. The characteristic Alfvén speed in these circumstances is very high indeed (perhaps several thousand km s^{-1}) and one can expect that, for a short time at least, reconnection takes place very rapidly with large inductive electric fields existing in the vicinity of the neutral line and voltage drops of ~ 1 MV. The situation is rather similar to that found in laboratory reconnection experiments where the reconnection rate is observed to suddenly increase, with a corresponding enhanced production of energetic particles, at the time when magnetic field lines carrying low plasma densities reach the neutral point¹². Note that these electric fields are not directly observable at ionospheric levels, however, one is naturally led to associate the neutral line with a type of auroral arc which moves rapidly polewards during the expansion phase of substorms^{13–15}.

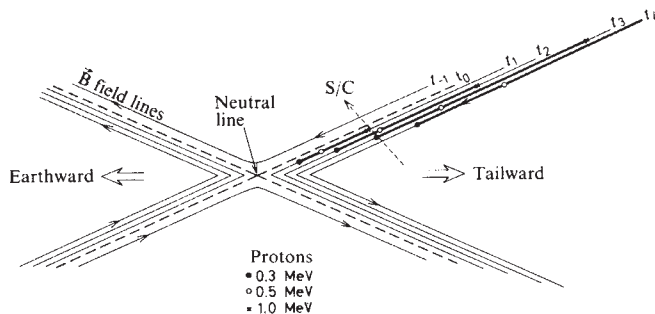


Fig. 2 Schematic diagram of the motions of magnetic field lines and the associated accelerated energetic particles resulting from magnetic reconnection. The positions of particles released from the neutral line at t_0 and at progressively later times t_1 , t_2 , t_3 and t_4 are shown more or less to scale, as is the motion of the spacecraft relative to the whole configuration.

In this context the inverse velocity dispersion may be interpreted as follows: it is assumed that particles on a given field line are accelerated more or less simultaneously in a small region surrounding the neutral line as the field line becomes reconnected (that is at the time it reaches the neutral point). Following reconnection, the magnetic field lines carrying this population of energetic particles move tailwards and earthwards away from the neutral line and on the tailwards side, the particles stream freely away from the Earth into interplanetary space. A schematic of the motions of magnetic field lines and the energetic particles is shown in Fig. 2. Reconnection of a particular magnetic field line and the associated particle acceleration is assumed to occur at time t_0 . At subsequent times (t_1 , t_2 , t_3 , ...) this magnetic field line moves away from the neutral line while the energetic particles it carries disperse along the line such that the faster particles are at any time further away from the source. During an outbound crossing of the plasma sheet boundary by a spacecraft (which is the case here, since the plasma disappears from the vicinity of the spacecraft) the magnetic field lines should appear as almost perfect 'velocity selectors' such that the lower energy particles are observed on the inner lines and the higher energy particles on the outer lines with respect to the plasma sheet. Thus particle detectors on the spacecraft could in principle detect a sequence of mono-energetic pulses with the energy increasing in time at a rate depending on the speed of the field lines and the motion of the plasma sheet boundary relative to the spacecraft. With finite energy and time resolution and a finite source size, the pulses must, of course, have finite width, but the dispersion effect will not be changed. In the present example, we estimate from the dispersion and the width of the pulses that the speed of reconnection is of the order of a few times the speed of the boundary motion, if the reconnection point is at a distance of $\sim 20 R_e$ from the Earth.

How do these particles become energised? There is mounting evidence that much of the energy dissipated resistively in the reconnection process goes into the production of high energy particles^{16–18}. The rather pronounced dawn-dusk asymmetry^{2,7,19,20} suggests a simple cross-tail acceleration mechanism associated with magnetic field reconnection^{21–23}. In this case one might expect the high energy channels to be dominated by highly charged species (for example O^{6+} and Fe^{9-13+} from the solar wind, if available) and this does not seem to be in agreement with the observations. However, if the reconnection takes place at a distance of the order of $20 R_e$ from the Earth it is likely that the plasma involved is of ionospheric origin and therefore singly charged. It is also possible that there could be local electrostatic acceleration in the vicinity of the neutral point, resulting from the fact that the electrons are constrained to move with the magnetic field lines, whereas the ions become 'non-magnetic' and hence the necessity for charge neutrality might require that the ions be ejected from this region. This would produce a similar energy per charge signature to the cross-tail acceleration mechanism, but the dawn-dusk asymmetry could not be easily explained. The latter seems also to be a difficulty for any

stochastic mechanism as does the potentially large gyro-radii of the ions and the very high anisotropy which is produced. Finally, pulses with the reversed velocity dispersion should be observed when the plasma sheet envelopes the spacecraft during the 'thickening', which takes place at a later stage in the substorm. We expect, however, that the voltage drops prevalent at such times are much smaller than during thinning and hence that the particle bursts should be detectable only by instruments with lower energy thresholds than described here.

We thank Dr S. M. Krimigis for making available data from the Johns Hopkins University, Applied Physics Laboratory experiment on IMP-7, and Dr L. A. Frank, Dr G. C. Gloeckler, and Dr N. F. Ness for results of experiments on the same spacecraft.

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Temperature dependence of atmospheric absorption in the wavelength range 8–14 μm

THE Earth's atmosphere has relatively high transparency to wavelengths between 8 and 14 μm , which makes them particularly important for communications and remote sensing. A reliable model of molecular absorption in this range is of particular value¹ as it may be used to relate atmospheric temperatures to radiant flux measurements made by artificial satellites—it seems that even in conditions when particle scattering can be neglected, absorption losses can be greater than is expected from present models. To study the remaining loss, or anomalous absorption, we have made new measurements in which particular attention was given to the temperature dependence of the anomalous component. The motivation for this was to test the hypothesis² that anomalous absorption could be ascribed to an equilibrium concentration of water dimers in the atmosphere. We find that this explanation cannot account for anomalous absorption in all conditions.

Measurements were made on a horizontal column of atmosphere 1.5 m above ground level and 560 m in length. The spectral observations were supplemented by laser measurements at wavelengths of 10.6 and 0.63 μm , the latter being used to quantify visibility. Standard meteorological determinations of air temperature and relative humidity were also made. A total of ~2,000 h of usable observation in various weather conditions was obtained between January and September of 1977. We have limited this discussion to conditions when the visibility was greater than 40 km so that the infrared absorption we measured

would not be complicated by particle attenuation, of which the upper limit could be estimated from the visibility.

The first step in the analysis was to separate out the water monomer component which is predictable from the measured humidity and temperature. The input to these model calculations is summarised in Table 1 and a representative spectrum is shown as curve $m-m'$ in Fig. 1. The values of anomalous absorption were then derived by subtracting predictions from observations. Contours were formed by taking average values for each of the 15 low absorption regions (windows) shown at the top of Fig. 1 and joining up their absorption ordinates at the mid-wavelength points, the windows being chosen to exclude most of the water monomer line centres.

The attribution of anomalous absorption to water dimers rests entirely on the observed temperature dependence being in agreement with that expected from the known intermolecular binding energy of the dimer. To test the hypothesis, therefore, we used equation (1) to reduce the field observations to standard conditions of density and temperature.

$$\alpha = Cd^2 \exp(B/k\theta) \quad (1)$$

α is an absorption coefficient in dB km^{-1} ; d is the measured water vapour density; B is a temperature exponent in eV per molecule, k is Boltzmann's constant in eV per molecule per deg K; θ is the temperature in K; C is a constant containing the relevant absorption cross sections which are assumed to be independent of temperature.

The values of B were then calculated for each window wavelength and the data were also separated into low and high temperature blocks to see if the exponents were themselves dependent on temperature. The division was made at 286 K to give about equal amounts of observation in the two ranges

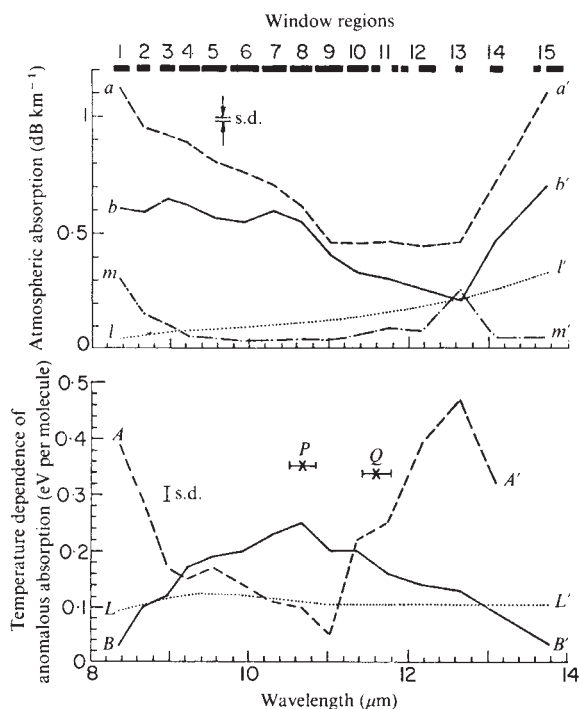


Fig. 1 Curves $a-a'$ and $b-b'$ show observed anomalous absorption values for mean temperatures of 281 and 290 K respectively, reduced to a water vapour density of 4.8 g m^{-3} . The estimated error is $\pm 0.02 \text{ dB km}^{-1}$. Curves $A-A'$ and $B-B'$ are the corresponding temperature dependence values expressed as the exponent B in equation (1), with a maximum estimated error of $\pm 0.04 \text{ eV per molecule}$ (at $12.6 \mu\text{m}$ wavelength). Curve $l-l'$ shows laboratory values from ref. 3 scaled to 290 K and density 4.8 g m^{-3} , and $L-L'$ is the corresponding laboratory temperature dependence. Curve $m-m'$ shows a monomer model spectrum from the data in Table 1, for 290 K and density 4.8 g m^{-3} . The values given by P and Q are taken from ref. 1, and apply to the temperature range 258 to 299 K.

Table 1 Sources of data used to compute theoretical absorption spectra of monomeric water vapour and carbon dioxide in the range 8–14 μm (1,250–710 cm^{-1}) and estimates for scattering

Molecular absorption model	
Line shape	Gross ¹² as formulated by Zhevakin and Naumov ¹³ and with corrections noted by Emery ¹⁴ .
Centre frequencies } Line intensities }	McClatchey <i>et al.</i> ¹⁵ .
Air broadened line widths	Benedict and Kaplan ¹⁶ .
Criteria for inclusion in the computation	
I 601 H ₂ O lines in the interval 710–1,250 cm^{-1} 849 CO ₂ lines in the interval 710–1,250 cm^{-1}	Lines which, at their peak, contribute 0.004 dB km^{-1} or more absorption for 4.6 torr water vapour pressure (or 330 p.p.m. CO ₂) when broadened by 1 atm. of air at 273 K.
II 16 H ₂ O lines in the interval 500–710 cm^{-1} 377 H ₂ O lines in the interval 1,250–1,800 cm^{-1} 358 CO ₂ lines in the interval 0–710 cm^{-1}	Lines which contribute 0.0004 dB km^{-1} or more absorption at the edges of the 710–1,250 cm^{-1} band in the above condition. In this category there are no H ₂ O lines above 1,773 cm^{-1} and no CO ₂ lines above 1,250 cm^{-1} . No H ₂ O lines below 500 cm^{-1} were included.
Scattering model	When the visibility is >40 km the 0.63 μm attenuation is <0.4 dB km^{-1} . For all infrared wavelengths we have assumed a scattering component of one-tenth of the value at 0.63 μm wavelength. The average expected spectral dependence for the scattering component shows a monotonic decrease from its value at 8 μm to half the value at 14 μm wavelength.

which were specified by their mean values of 281 K and 290 K respectively.

Figure 1 shows that the measured anomalous absorption (curves $a-a'$ and $b-b'$) is on average several times greater than both the monomer model absorption (curve $m-m'$) and the excess absorption which was measured in the laboratory by Burch³ (curve $l-l'$). The spectral shapes $a-a'$ and $b-b'$ are also different from both the model and laboratory results. In the absence of a theoretical prediction of the strength and shape of the dimer spectrum at these wavelengths, such results by themselves give little insight to molecular models. The variation of absorption with temperature, however, is predicted by the dimer model to have a temperature exponent B equal to the intermolecular binding energy and this can be determined independently. Values from 0.12 to 0.16 eV per molecule are given from two different sources. One is derived from the temperature dependence of the second virial coefficient⁴ and the other comes from *ab initio* calculations of the total electronic binding energy and the change in vibrational zero point energy on dimerisation^{5,6}. Figure 1 shows that while the temperature exponent of absorption measured in the laboratory by Burch (curve $L-L'$) can reasonably be identified with the equilibrium dimer binding energy, the values which we measured in the real atmosphere (curves $A-A'$ and $B-B'$) cannot. These are on average significantly higher, with the greatest divergence between laboratory and field results appearing at the lower mean temperature. We conclude from this that an explanation of anomalous absorption being due to water dimers for all atmospheric conditions is untenable.

In relating our results to those of other workers, Coffey¹ provides a useful survey of available data. It also includes new observations which give an even higher temperature dependence as shown by the points P and Q in Fig. 1, and it is surprising therefore that Coffey concludes that they support the equilibrium dimer hypothesis. The discrepancies between temperatures determined radiometrically from satellite observations and those made '*in situ*' also suggest that a dimer model is inadequate⁷. We note that some of the discrepancies reported are greatest at low temperatures⁸ which is in line with the present results.

No alternative molecular model is yet available to explain anomalous absorption. As a pointer to possible mechanisms we note that there are other results for water vapour which show anomalously high temperature exponents at low temperatures. These are from measurements of the speed of sound⁹ and of absorption¹⁰ in the region of 7 cm^{-1} . However, as such steep temperature dependence is not predicted from equilibrium properties, we propose that the phenomena must be attributed to non-equilibrium molecular complexes of water. There is some support for this notion in the fluctuations of absorption

which appeared in our measurements. These were about three times greater than could be attributed to detector noise and were too large to be caused by refractive angular scintillation. The results suggest that the dissipative absorption may itself be a time-dependent quantity whose relaxation is responsible for the fluctuations. Such variability has been reported elsewhere¹¹.

Our results show that new molecular phenomena may have to be considered in the search for a better understanding of 10- μm region absorption and the importance of satellite radiometry alone points to the pressing need for this. If, as we suggest, non-equilibrium phenomena do play a part, this will complicate modelling but may bring some benefit, as it suggests that a systematic study of absorption fluctuations could lead to an improved understanding of the underlying physics.

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Effects on prolific petroleum source rocks and major coal deposits caused by sea-level changes

THE nature and abundance of planktonic organic matter—the main source of crude oil—and moreover its preservation in sediments are affected by sea-level changes. Deposition of paralic coals might be also influenced. It is shown here that these

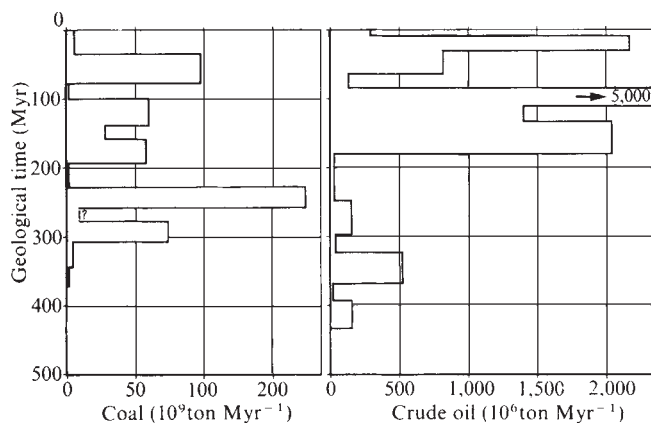


Fig. 1 Distribution of coal and petroleum as a function of geological time. The surface of the block areas is proportional to the total resources (in place, not necessarily recoverable) in geological series of each period. Data on coals, from Strakhov¹, are related to world reserves. Data on crude oil, from Bois *et al.*² are related to 148 petroleum zones, amounting to ~85% of the world resources. The age plotted for crude oil is the age of the source rock.

considerations would explain large accumulations of coal or petroleum source rocks over relatively short periods of time.

Available evidence indicates that coal and petroleum are unevenly distributed among geological periods. Figure 1 shows the time distribution of coal (world reserves¹) and petroleum source rocks. The latter is plotted in terms of total—not necessarily recoverable—oil stored in reservoirs derived from the source rock. The plot includes 148 petroleum zones (ref. 2 and C. Bois, personal communication) of the world amounting to 400×10^9 tons of oil in reservoirs. Prolific petroleum source rocks, deposited over part of Jurassic and Cretaceous (from 180 to 85 Myr) are responsible for 70% of the crude oil in these 148 petroleum zones; however, this interval amounts to 17% only of the time elapsed since the Precambrian. Considerable accumulations of coal occurred during Middle and late Carboniferous, Middle and Upper Permian, Lower and Middle Jurassic, Lower Cretaceous, and uppermost Cretaceous to Eocene. These intervals amount to 30% only of the time elapsed since the Precambrian, but they integrate ~95% of the world reserves of coal.

Organic geochemical studies have shown that source rocks able to generate large quantities of oil have specific characteristics. They contain an abundant organic matter of planktonic and/or bacterial origin, preserved and deposited in a reducing environment³. The resulting kerogen belongs to types I or II

defined by Tissot *et al.*⁴ on the basis of elemental analysis and infrared (IR) spectroscopy. However, the cause for accumulating large quantities of this material over wide areas during certain periods of time was not clear.

Recent developments in marine geology and geophysics made possible a comparison of these events with global sea-level changes. Pitman⁵ showed that absolute rise or fall of sea-level are caused by the volume changes of the mid-oceanic ridge system. These changes are mainly due to change in spreading rate and to creation or destruction of a ridge system. The sea level (curve E, Fig. 2) was ~+350 m above present in the late Cretaceous (85 Myr) and fell to +60 m in Middle Miocene (15 Myr). Since that time glacial fluctuations may have dominated sea-level changes. Vail⁶ studied the relative changes of sea level, that is, its rise or fall with respect to land surface, from the onlap of coastal deposits in seismic records of maritime sequences (curve R, Fig. 2). Both curves E and R show a good agreement, with respect to general trend and average value. Thus, it is possible to extend curve E from Cretaceous to Palaeozoic by E' (averaging the general trend of R): two major cycles⁶ of sea-level rise and fall occurred from Cambrian to Triassic (570–200 Myr) and from Jurassic to present (200–0 Myr).

Productivity and composition of marine phytoplankton—the main primary producer of marine organic matter—seems greatly influenced by these global cycles. An estimation of its total abundance shows two distinct maxima (Fig. 2) corresponding to the highest sea level, the largest extension of epicontinental seas, which are more productive than deep oceans³. Furthermore, the first cycle was governed by organic-walled plankton (acritarchs, green and blue-green algae), whereas the second cycle was dominated by calcareous nannoplankton (coccolithophorids, dinoflagellates) and later siliceous plankton (silicoflagellates, diatoms³). Dominant epibenthic faunas⁸ were also replaced. On land, the disappearance of lowland plains in late Cretaceous time (sea level 350 m above present) may also be responsible for some dramatic changes of fauna.

Preservation of organic matter is a crucial point for deposition of rich source rocks. The main factors of destruction of this material are feeding heterotrophic animals and biochemical degradation by aerobic bacteria: thus the absence of oxygen in waters and in freshly deposited sediments is the main condition for preservation⁹. Shallow epicontinental seas transgressive over continental depressions often fulfill this requirement. The sea is surrounded by emergent lands, providing mineral nutrients and favouring algal blooms. In turn, the blooms resulted in an abundant bacterial consumption of oxygen and provided temporary anoxic conditions in the landlocked sea, where waters were not sufficiently renewed¹⁰. In deeper waters, anoxic

Fig. 2 Global cycles of absolute (E) and relative (R) sea-level changes, according to Pitman⁵ and Vail⁶, and their influence on marine and terrestrial populations. Estimated abundance of phytoplankton⁷ shows two maxima corresponding to the highest sea-level positions. Its dominant composition³ changes from one cycle to the other, and also that of epibenthos⁸. Terrestrial faunas⁸ are also drastically changed after the highest sea-level of late Cretaceous.

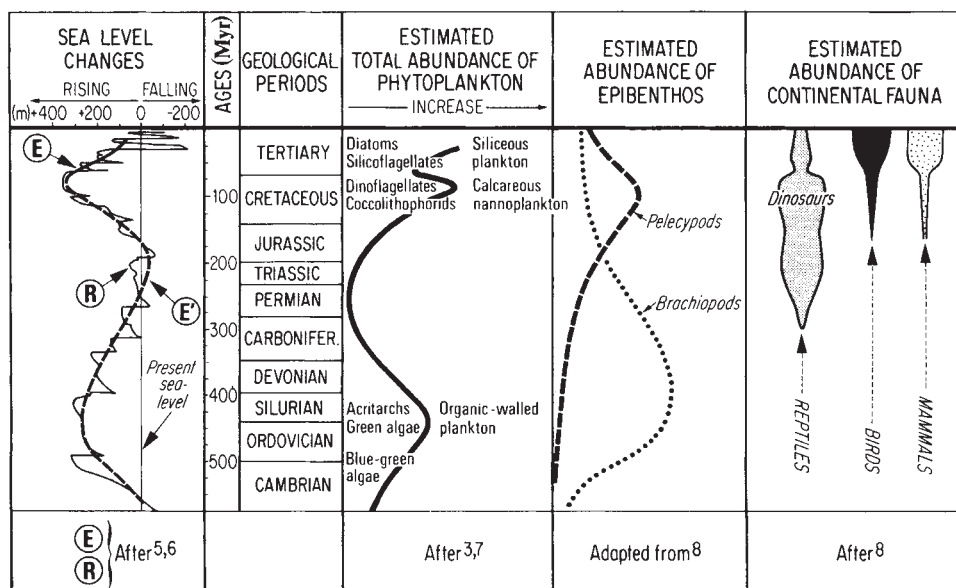
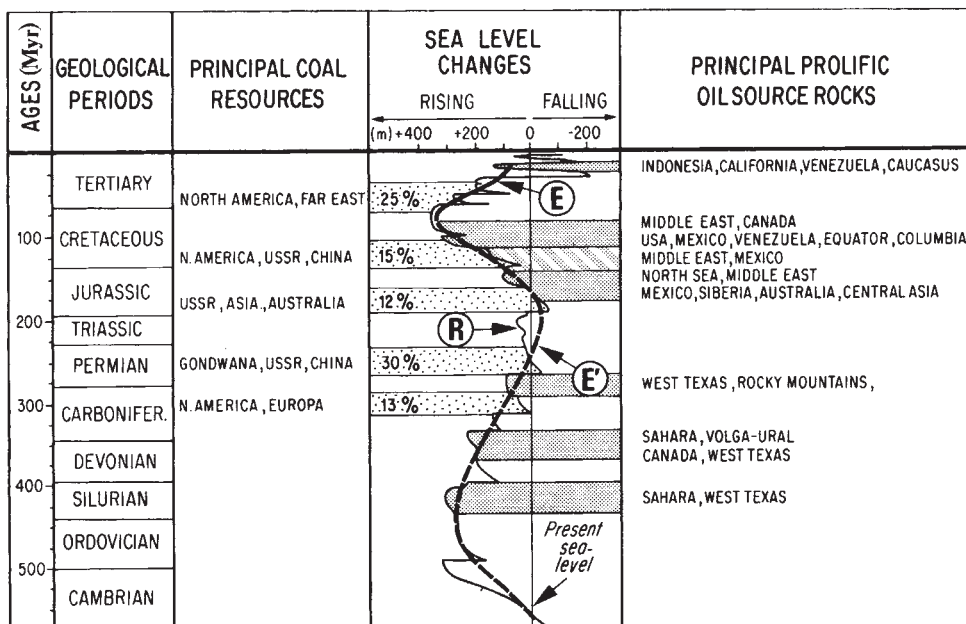


Fig. 3 Principal periods of deposition of prolific oil source rocks and major coal deposits are compared with worldwide transgressions and regressions. The periods considered for petroleum source rocks are responsible for more than 10^8 tons of oil Myr^{-1} during the first cycle (570–200 Myr) and more than 10^9 tons Myr^{-1} during the second cycle (200–0 Myr).



basins, such as the Black Sea, or areas associated with upwelling may provide comparable conditions.

Worldwide transgressions over the continental platform have been observed. Transgressions and regressions result from the balance of the rate of sea level rise or fall and the rate of subsidence minus sedimentation rate. Thus worldwide transgressions may be caused by changes of the rate of sea level rise or fall⁵. The second-order cycles of curve R (Fig. 2) illustrate these events⁶: a transgression appears as a rise of R with a positive departure compared to E–E'; a regression appears as a fall of R with a negative departure. The comparison of these cycles with the major occurrences of prolific oil source rocks (Fig. 3) indicates that they were mainly deposited during periods of global marine transgressions. For instance, the Silurian transgression deposited the rich source rocks of the Sahara over the glacial topography inherited from the Ordovician continent. In Western Europe, the successive Jurassic transgressions over the Triassic continent deposited the source rocks of the North Sea and the Toarcian and Kimmeridgian oil shales (France, Germany, UK).

However, the potential for oil generation seems to be lower by about 1:10 in the older cycle, as compared to the younger one. This is probably due to a greater thermal maturation—generating gas—and to tectonic events (faults, folding and erosion) which have destroyed a large part of the oil originally formed.

Most important coal occurrences were formed by large accumulations of terrestrial higher plants in coastal or paralic basins. A long-lasting subsidence is necessary to provide thick series of shallow deposits and a moderate relief of the hinterland to avoid dilution by inorganic material. The most typical environment is provided by coastal lowlands with swamps which develop after regression of the sea. The comparison of the age of the major coal deposits— $> 50 \times 10^9$ tons Myr^{-1} —with the second-order cycles of curve R (Fig. 3) indicates a frequent correlation with worldwide regressive periods.

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Trace elements in the fluid phase of the Earth's mantle

IT has become apparent during the past decade or so that CO_2 is a significant component of the Earth's upper mantle. In addition to the indirect evidence including CO_2 emanations from volcanoes throughout the world and the abundance of CO_2 in kimberlites and carbonatites, it is now known that CO_2 is ubiquitously present in xenoliths of mantle material brought up in alkalic basalts and kimberlites. This latter point was first made by Roedder¹ who demonstrated that the fluid in optically visible inclusions is essentially pure CO_2 under pressures as great as 5 kbar at eruptive temperatures. Such pressures provide only a minimum depth of origin, because Roedder further showed that most of the inclusions he observed are secondary, that is, they occur as arrays on healed cracks. A more recent study² indicated pressures as high as 10 kbar in secondary inclusions. H.W.G. and Radcliffe³ found, using transmission electron microscopy (TEM), that submicroscopic fluid inclusions also occur within the crystals of mantle xenoliths, and that these bubbles have exsolved (precipitated) from solid solution. Thus, at least the CO_2 now present in submicroscopic fluid inclusions is indigenous to the crystals in which it is found, and probably represents the source of crack formation and filling to produce the secondary inclusions on the way to the surface³. The effect CO_2 -bearing phases have on the distribution of trace elements in the mantle and during partial melting is considered here.

The term 'fluid inclusion' is used here as a descriptive term only; I reject the usual connotation that the fluid has been 'included' into the crystals as a growth accident in an igneous environment. For example, virtually all of the rocks in question are metamorphic and show no signs of introduction of a fluid phase from an external source. Moreover, metamorphic recrystallisation 'excludes' fluid and concentrates it onto grain boundaries, rather than 'including' it during grain growth³.

CO_2 would appear, then, to be an important component of most, perhaps all, of the upper mantle and two questions are paramount: (1) in what phases does the CO_2 reside in the upper mantle?; and (2) what effect do these CO_2 -bearing phases have on the partitioning of trace elements within the mantle and between melt and residuum during partial melting? The former is easier to answer. There now seems to be too much CO_2 (at

least in the uppermost mantle) for it simply to be dissolved in the silicates as originally assumed by H.W.G.⁴; moreover, recent experimental work indicates that in the mantle beneath undisturbed continents, the CO₂-bearing phase is probably a carbonate⁵⁻⁷ and beneath oceans the stable phase may be a carbonate^{5,6} or supercritical fluid⁷. All the data strongly suggest, however, that because of the steeper geothermal gradient in the vicinity of an oceanic ridge, the stable phase in the lithosphere under ridges will be fluid CO₂. The second question is more difficult to answer, but is fundamentally more important because the concentrations of trace elements in melts derived from the mantle are routinely used to infer the present composition of the upper mantle and its geochemical evolution.

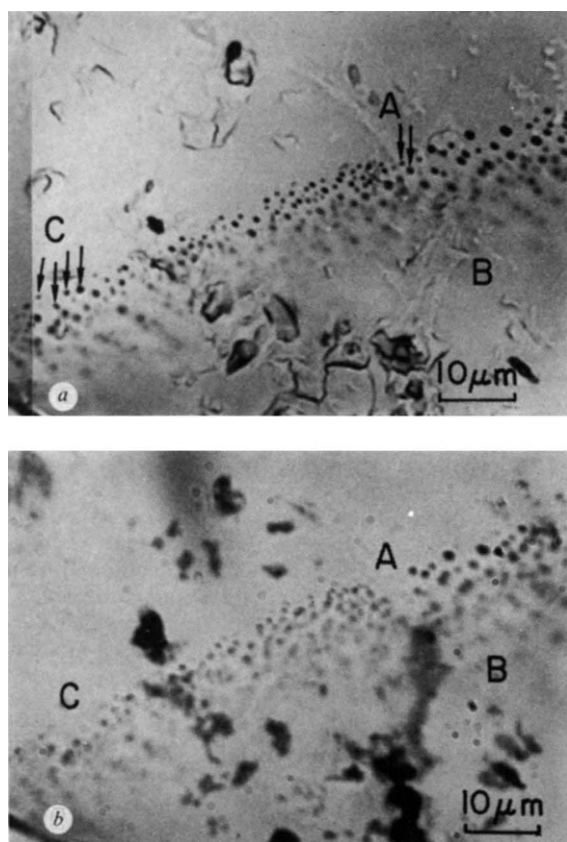


Fig. 1 Photomicrographs showing region of olivine specimen H-1 before (a) and after (b) ion probing. A healed crack decorated with 2-phase CO₂ bubbles intersects the top of the thin section along line AC. Arrows in (a) point to bubbles which were removed by sputtering and are absent in (b). Irregular black and grey areas in (b) are surface pits from which the gold film could not be removed after probing.

These inferences are always based on calculated or experimentally determined element partitioning between basaltic melt and mantle silicates, especially clinopyroxene and garnet. If significant quantities of these elements reside in another phase—a CO₂-bearing phase—and if portions of this phase are not incorporated into the melt, then current interpretations of trace element data could be in error. The ubiquitous occurrence of CO₂ inclusions in refractory xenoliths such as harzburgite and dunite indicates that melt extraction does not scavenge all of the CO₂-bearing phase.

As a first step to determination of the trace element problem, an ion probe study has been inaugurated to attempt detection of trace elements in CO₂ inclusions⁸. The procedure chosen was to 'drill' down through host crystal and break bubbles. To simplify the initial search, elements known to be major components of carbonatites and to have no good crystallographic home in mantle phases (olivine, orthopyroxene, clinopyroxene, garnet,

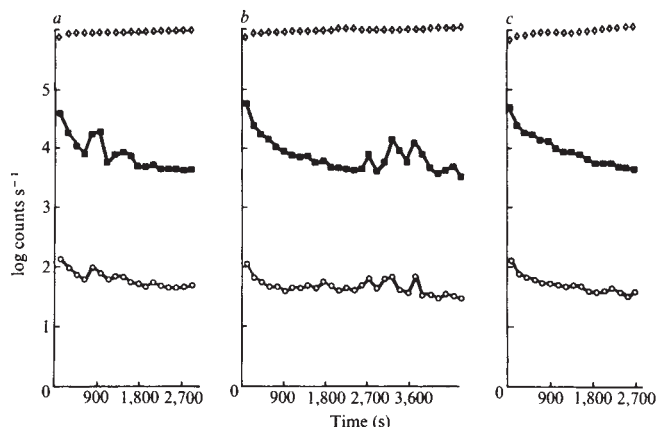


Fig. 2 Depth profiles for masses 24, 39 and 47 obtained from areas A, (a) C, (b) and B (c) of Fig. 1. Area B is a control profile obtained where no (optically visible) bubbles were present: ■, ³⁹K; ○, ⁴⁷Ti; ◇, ²⁴Mg.

spinel) were chosen as good candidates. In addition, mass numbers known to give interfering peaks produced by the matrix elements and polyatomic reaction products of matrix elements with the ¹⁶O beam were omitted. For example, ¹²C, ²⁸CO, ⁴⁴CO₂, ⁴⁰Ca, ⁴⁰Ar were not attempted due to interference with ²⁴Mg²⁺, ²⁸Si, ⁴⁴SiO and ⁴⁰MgO respectively. Five or six different mass numbers were searched in each run, including trace elements and elements to serve as internal monitors of the matrix, such as ²⁴Mg. The results were positive for masses 39(K) and 47(Ti), suggestive for mass 140(Ce) and inconclusive for masses 1(H), 85(Rb), 86(Sr), 87(Sr), 138(Ba), 142(Ce) and 208(Pb).

A portion of a bubble array on a healed crack in olivine of dunite specimen no. H-1 from the 1801 Kaupulehu lava flow of Hualalai volcano, Hawaii, is shown in Fig. 1a before ion probing and in Fig. 1b after probing. At site C, four inclusions were removed during bombardment of the surface for 80 min with a 15 nA ¹⁶O beam of elliptical cross-section (~8 μm × 15 μm). Mass numbers were examined sequentially with each mass counted for 5 s, except for ²⁴Mg which was counted for 3 s. The concentration profile produced during the erosion at site C is given in Fig. 2b. The four distinct peaks in masses 39 and 47 are presumed to correlate with the four bubbles removed by sputtering; no corresponding peaks (or valleys) in ²⁴Mg are observed.

Another profile measured at site A of the same inclusion array broke two bubbles (Fig. 1) and shows two peaks (Fig. 2a), whereas a control profile measured at site B in the same olivine crystal in an area of no fluid inclusions shows no peaks (Fig. 2c). Figure 3 shows similar results for an olivine crystal of lherzolite specimen No. SAL-3 from Salt Lake Crater, Oahu, Hawaii, and Fig. 4 shows the results of a long profile of an orthopyroxene crystal of this same specimen. The apparent noise in the mass 39 data of the latter profile is produced by breaking numerous small inclusions (≤1 μm diameter) and three larger ones (~5 μm diameter). Of particular interest in the mass 39 profile of Fig. 4 is the large, double peak between 6,200 and 7,400 s, and the secondary one between 7,800 and 8,500 s. These probably represent penetration of the three larger bubbles. These peaks correspond exactly to the highest values of the mass 140 profile which also shows a large, divided peak and a narrower one. Although the maximum count-rate is 4.5 c s⁻¹ for mass 140, this represents 2.9 standard deviations above the mean of all data points after surface contamination had been removed (2,700 s). Similarly, the two subsidiary peaks represent 1.8 standard deviations above the mean. Thus, the data can reasonably be considered significant on their own, and correspondence with the largest peaks in mass 39 increases the likelihood that these inclusions contain detectable amounts of cerium.

Quantitative interpretation of these data depend on the ionisation and collection efficiency of the ions involved, which, in

turn, critically depend on whether the elements in question are in the fluid itself or are present as a solid film on the surface of the bubbles. For potassium, for instance, the fraction of atoms detected from a fluid is of the order of 10^{-3} , but from a solid substrate is ~ 0.6 (T. Whatley, personal communication). Two lines of evidence strongly suggest that the trace elements detected here are in a surface film: (1) If the efficiency of detection is 10^{-3} , the K content of the bubbles in Fig. 2 is $>10\%$, an apparently unreasonably high value. On the other hand, if the detection efficiency is 0.6, the K content is about 1,000 p.p.m. of the fluid contents; (2) the peaks last too long. The best example of this phenomenon is the double peak in Fig. 4, which lasts for 20 min. During this time the beam will have eroded about $0.5\text{ }\mu\text{m}$ deeper into the pyroxene crystal and opened an orifice as large as $3\text{ }\mu\text{m}$ across on the $5\text{ }\mu\text{m}$ bubble(s). We can expect that the fluid, under very high pressure, would escape in the first few seconds of this time, hence the mass 39 and 140 elements cannot be in the fluid phase itself. It would seem most reasonable to conclude, therefore, that the trace elements detected in this study were originally in the fluid at high pressure and high temperature, but are now located in a film on the bubble walls; after penetration of the bubble, the ion beam 'washes out' the thin film.

Masses 39 and 47 which have been definitely detected here, are not uniquely interpreted as K and Ti; they could be ^{39}NaO and ^{47}PO . Na is unlikely to be a major contributor to the peaks reported here because the vast majority of Na is always registered at mass 23. A significant contribution of Na at mass 39 would therefore imply a very large Na content to the inclusions, a composition inconsistent with the conventional fluid inclusion studies^{1,2}. ^{31}P , however, is commonly detected as ^{47}PO with an ^{16}O beam (T. Whatley, personal communication) and hence may be a significant, even dominant contributor to the peaks at mass 47. In this regard it is important to distinguish the results of this study and the analysis of glassy rims sometimes found on fluid inclusions in these rocks. Although K, Na, Ti and P have been detected in such rims², the surface film detected here seems to be a different phenomenon. Most inclusion arrays in peridotite xenoliths contain only fluid. This has been determined by optical observation of the brownian motion of the inner vapour bubble when the inclusions are sufficiently large ($\geq 2\text{ }\mu\text{m}$ diameter), and by TEM when they are not. In every array investigated, when all of the large inclusions show an inner bubble in motion and no glass rim, the TEM confirms the absence of a glass rim on the smaller inclusions. Some submicroscopic inclusions, however, do suggest a very thin ($<100\text{ }\text{\AA}$) surface film, which I have so far been unable to convince myself was definitely present. Furthermore, the three larger inclusions originally present in specimen SAL 3-14 (Fig. 4) displayed moving vapour bubbles

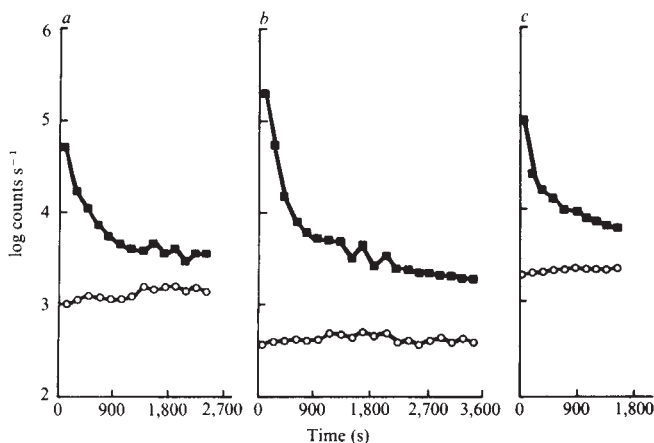


Fig. 3 Depth profiles for masses 39 and 47 for three areas of specimen SAL-3: *a*, SAL-3-14A; *b*, SAL-3-26A; *c*, SAL-3-25A. Profile 25A is a control obtained where no (optically visible) bubbles were present. ■, ^{39}K ; ○, ^{47}Ti .

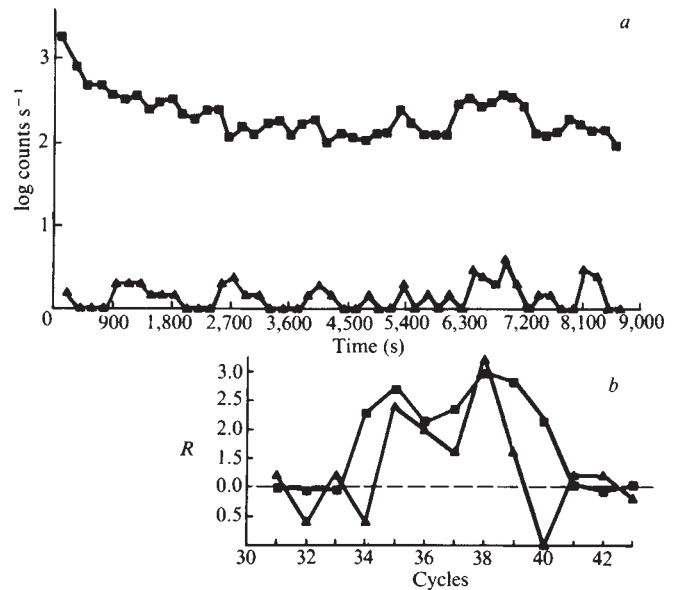


Fig. 4 Depth profile for masses 39 and 140 for area 14 of specimen SAL-3. Large doublet, peak between 6,200 and 7,400 s (cycles 33–43) is replotted in (*b*) in terms of the ratio (*R*) of counts s^{-1} divided by the average counts s^{-1} for cycles 33–43. ■, ^{39}K ; ▲, ^{140}Ce .

and lacked a glassy rim. The volume fraction of fluid is thus very much larger in the inclusions described here than in those with glassy rims figured by Roedder¹ and Murek *et al.*², and it would seem difficult to interpret my results as evidence of an immiscible silicate liquid incorporated along with the CO_2 . It seems more likely that these elements were dissolved in the fluid at high pressure.

The results of this study demonstrate that the CO_2 -bearing phase in the mantle contains significant quantities of cations other than carbon. They do not, however, help much in determination of whether the CO_2 was already a fluid or was in a carbonate when it began its trip to the surface (because the inclusions analysed are secondary and could conceivably represent the product of carbonate breakdown). However, other workers have shown that the CO_2 bubbles in xenoliths also contain significant quantities of noble gases^{9–11}. Of major interest is the detection of excess ^{129}Xe (ref. 11) with its implication that the Earth formed within 76 Myr of nucleosynthesis¹² and has not been totally outgassed since that time^{11,12}. Another point of major importance is the large quantity of ^{40}Ar , giving anomalously old K/Ar ages. These latter observations suggest that the CO_2 -bearing phase has acted as a sink for rare gases—a property which is more likely to characterise a fluid than a carbonate.

Finally, the detection of alkalis and a suggestion of light rare earths suggest caution in interpretation of mantle compositions from basalt chemistry using silicate-liquid partition coefficients.

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Speciation of dissolved iodine in estuarine waters

IODINE in seawater is present as iodate (IO_3^-) and as iodide (I^-)¹⁻³. Although there is some uncertainty in the true redox potentials of natural waters⁴, the thermodynamically stable species of iodine in seawater, and in river waters which are well oxygenated, is iodate⁵⁻⁷. We have studied an estuary to determine the distribution of iodine species as a function of salinity, and examined the results for evidence of interconversion of species in the estuary. We report here that iodide is the dominant form in river water and is oxidised to the thermodynamically stable iodate in the sea, not in the estuary.

Our study area was the estuary of the Yarra River, which drains part of southern Victoria, Australia, and includes the city of Melbourne with 1,200,000 inhabitants in its catchment. The estuary is 22 km long and highly stratified⁸, but not oxygen deficient. The river discharges into Port Phillip Bay, a large embayment with only a small outlet to Bass Strait. The survey was made in late winter at a time of high river flow (22 August 1978). Water samples were filtered through prewashed Gelman 0.45 μm membrane filters, stored in glass bottles at 4 °C in the dark, and analysed within a month of collection. Previous storage experiments showed that no measurable changes in iodate or iodide occurred in water stored in these conditions.

Iodate in seawater, and in estuarine waters of salinity >2‰ was determined directly by differential pulse polarography (DPP)^{9,10}. A 20-ml aliquot of the water sample was pipetted into a polarographic cell, EDTA solution was added to suppress the zinc wave, and the solution deoxygenated by bubbling argon. A Metrohm E500 series Polarecord with a dropping mercury electrode, platinum wire counter electrode, and Ag/AgCl/M KCl reference electrode, was used. Concentration of iodate was determined by the method of standard additions. Iodide was determined in a separate aliquot of the sample by oxidation of iodide to iodate by addition of chlorine water¹¹, evaluation of the new level of iodate by the polarographic method, and calculation of iodide by difference. We found ultraviolet (UV) irradiation¹⁰ unsuitable for iodide determination, as it produced peroxide that interfered and also gave iodate from 'organo'-iodine compounds in estuarine waters. No interfering species were produced by chlorine water in the experimental conditions used. In river water, the iodate-plus-iodide concentration could not be found by simple addition of chlorine water, as the large amount of dissolved organic matter interfered with the determination. A modified procedure was developed, in which a 50-ml aliquot of river water was treated with sodium hydroxide and chlorine water, then evaporated to dryness under an infrared lamp. The residue was redissolved in 10 ml of doubly distilled, deionised water, this solution treated with chlorine water, and sodium formate solution added. The resulting solution was filtered to remove coagulated organic matter and made up to 25 ml with water. Iodate-plus-iodide was then determined on a 20-ml aliquot after addition of EDTA. Analysis of water of salinity 5‰ by both procedures yielded results that were not significantly different, indicating that the more complex treatment did not convert any further iodine to iodate.

Replicate analyses of a bulk estuarine sample of salinity 19.4‰, collected at an earlier date, gave 7.9 $\mu\text{g l}^{-1}$ I as iodate with a coefficient of variation of 6.7%; 53.4 $\mu\text{g l}^{-1}$ I as iodate-plus-iodide with a coefficient of variation of 3.2%; and by difference, 45.5 $\mu\text{g l}^{-1}$ I as iodide with a coefficient of variation of 9.9%. The limit of detection of iodate is largely dependent on establishment of the baseline of the polarograms, but taking two standard deviations at the 7.9 $\mu\text{g l}^{-1}$ level it is about 1.0 $\mu\text{g l}^{-1}$ I. Salinity was measured using an AutoLab 602 salinometer calibrated using IAPSO standard seawater.

Results from the Yarra River estuary (Fig. 1) indicate that both iodide and iodate show conservative behaviour. The river

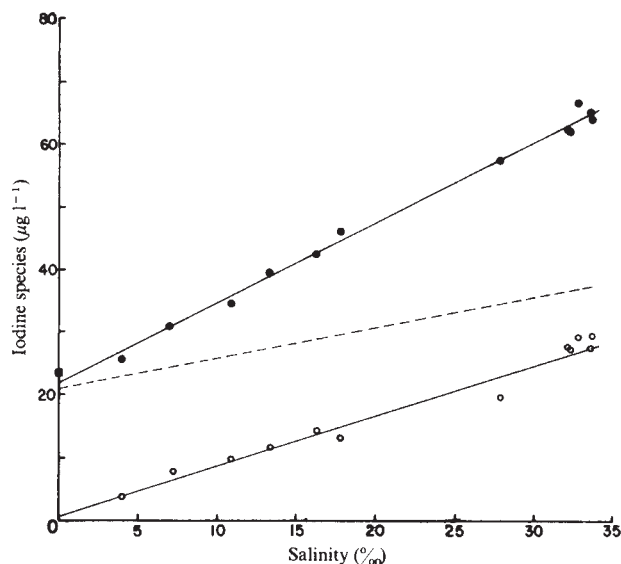


Fig. 1 Relationship between salinity and concentration of iodate (○) and iodate-plus-iodide (●). Lines of best fit are shown, and the dashed line represents the iodide concentration determined by difference. The concentration of iodate-plus-iodide in river water (■) was determined by a procedure different from that used for estuarine waters.

water contained 23 $\mu\text{g l}^{-1}$ I as iodide, and <1.0 $\mu\text{g l}^{-1}$ as iodate. The seawater contained 28 $\mu\text{g l}^{-1}$ I as iodate, and 36 $\mu\text{g l}^{-1}$ as iodide, as the average of five samples with a mean salinity of 34.0‰. 'Iodine', as iodide-plus-iodate, determined after oxidation with chlorine water, was 23 $\mu\text{g l}^{-1}$ in the Yarra river water which is within the range of 2.2 to 42.4 $\mu\text{g l}^{-1}$ reported for iodine in other rivers of the world¹². The dominant form of iodine in rocks and soils is iodide. This is likely to be the case in the Yarra drainage basin, and apparently no conversion to iodate occurs in the transit from source mineral to the river. 'Iodine' in seawater of 34‰ salinity from Port Phillip Bay at 64 $\mu\text{g l}^{-1}$ I is higher than seawater values reported by Truesdale¹³, but comparable with the average values of Barkley and Thompson³ and Wong¹⁴.

The present study indicates that there is no measurable conversion of the iodide of river water to iodate within the Yarra River estuary, and that the iodate in the estuary comes from the seawater. Consequently, it seems that oxidation of iodide to the thermodynamically stable iodate occurs in the seawater outside the estuaries. Two mechanisms, which are known to effect oxidation of iodide to iodate occur through UV irradiation, and by biological activity of algae. We have used UV irradiation for conversion of iodide to iodate as an analytical technique, and found that quantitative conversion of iodide to iodate required ~3 h exposure to a 1,200 W mercury lamp at a distance of 6 cm. Sunlight would be expected to affect only the surface water, and then at a very slow rate. The effects of algae on iodide and iodate in seawater have been reported. Sugawara and Terada¹⁵ found that a diatom culture in seawater containing 16 $\mu\text{g l}^{-1}$ iodide converted 3 $\mu\text{g l}^{-1}$ iodide to iodate in four weeks. Truesdale¹³ found that some *Chlorophyceae*, cultured in seawater containing 50 $\mu\text{g l}^{-1}$ interconverted not more than 5 $\mu\text{g l}^{-1}$ in an unspecified time.

Although we did not observe any apparent reaction of iodide or iodate in the estuary, we did detect the presence of a third iodine form. When we exposed waters of intermediate salinity to UV irradiation for 4 h before addition of chlorine water, we observed an increase in the iodate detectable by DPP compared with that found after oxidation using chlorine water alone. Our experiments with 2-iodo-benzoic acid in the conditions used in analysis of estuarine waters showed that iodide in this compound was not converted to iodate by treatment with chlorine water, whereas UV irradiation followed by treatment with chlorine

water gave quantitative recovery of the organo-iodine as iodate determined by DPP. The estuarine water of salinity 13.4‰ contained $27.9 \mu\text{g l}^{-1}$ I as iodide, $11.6 \mu\text{g l}^{-1}$ as iodate, and a further $7.0 \mu\text{g l}^{-1}$ released after UV irradiation and treatment with chlorine water. The increase is probably the result of decomposition of organo-iodine compounds by the UV irradiation, and 15% of the total iodine was present in this estuarine water as 'organo'-iodine. The seawater of 34‰ did not yield additional iodate when UV irradiated before chlorination. Organo-iodine has been reported in seawater by Truesdale¹⁶ but there are no previous reports of iodine speciation in estuarine or river waters.

The results of Truesdale¹³ show a relatively constant 'total' iodine in seawaters, with a decrease in iodide and increase in iodate progressing from Menai Strait, through the Irish Sea, to North Atlantic Ocean waters. He suggests that iodate-reducing substances introduced to the sea by terrestrial run-off might explain these observations.

On the basis of our evidence, variations of iodide to iodate ratio might also be largely explained as the result of oxidation of iodide delivered by rivers to the oceans. The production of iodate seems to be thermodynamically favoured, but limited by the slow rate of conversion, with a reported half life of tens of years¹⁷. This is too slow for it to be observed in the Yarra River estuary, where residence time is only days. Hence conversion of iodide to iodate must occur in the ocean, possibly under the influence of enzyme systems.

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Effect of glacial ice melting on the Antarctic Surface Water

MOST glacial ice melting occurs below the sea surface and in regions where ice shelves and icebergs are concentrated, as along the Antarctic coastline. We show here that this melting can significantly affect the Antarctic Surface Water (AASW) chemistry and biology only for glacial ice constituents are greatly depleted (for example, salt, oxygen-18) or enhanced (for example, ammonia?) relative to the ocean. The surface water nutrient (nitrate, phosphate, silicate) budget is controlled primarily by the upwelling of deep water. Melting beneath the Ross Ice Shelf results in the northwards lateral movement of near-freezing water to the continental shelf break. Basal melting of icebergs and smaller ice shelves may contribute to the temperature minimum at the base of the AASW.

The volume of meltwater produced annually from melting Antarctic glacial ice will be three orders of magnitude less than the AASW reservoir, which extends from 50 to 200 m depths over the $20 \times 10^6 \text{ km}^2$ area south of the Polar Front (Antarctic convergence). For an Antarctic ice cap in an equilibrium state, the average annual volume of glacial ice lost must equal the 0.15 m yr^{-1} net accumulation over the $14 \times 10^6 \text{ km}^2$ area of Antarctica¹. We assume relatively little ablation². Net deep melting of 0.30 m yr^{-1} (refs 3, 4) beneath the large ice shelves, which comprise less than 11% of the ice cap^{4,5}, would account for no more than a quarter of the $2.1 \times 10^3 \text{ km}^3$ wastage. The remaining ice will melt at shallower levels, within or near the base of AASW from icebergs, ice streams, small ice shelves and from the northern edges of the major ice shelves.

Parker *et al.*⁶ have suggested that nitrogen-laden ice originating on the Antarctic continent provides, on melting, a major contribution ($7\% \text{ yr}^{-1}$) to the nitrogen budget of the 50 m epipelagic zone of the Antarctic oceans. Since then Biggs has shown⁷ that Parker *et al.* used incorrect nitrate ($\text{NO}_3\text{-N}$) values, and he has recomputed the nitrate input from continental ice as 0.02% of that in the AASW, assuming a surface mean of $12 \mu\text{g-atoms l}^{-1}$. However, there are significant differences between the nitrate observations reported by various expeditions in the Antarctic oceans (Fig. 4 in ref. 8). Our AASW nitrate measurements^{8,9} and those of the Geosecs expeditions¹⁰ range from 15–30 $\mu\text{g-atoms l}^{-1}$, with a mean around 22 $\mu\text{g-atoms l}^{-1}$. With this higher nitrate mean, and a reservoir thickness closer to 100 m than to 50 m, the net nitrate input from glacial ice would be 0.005% of that already in the AASW.

The Parker *et al.*⁶ nitrate mean of $19.57 \mu\text{g l}^{-1}$ ($1.4 \mu\text{g-atoms l}^{-1}$) is an order of magnitude less than the AASW nitrate concentration, so iceberg melting would cause a slight nitrate decrease locally in the AASW rather than an enrichment as claimed. Subsequently, Parker *et al.*¹¹ reported nitrate values as high as 37 $\mu\text{g-atoms l}^{-1}$ in additional ice samples, but they do not indicate whether their nitrate mean has increased or decreased from $1.4 \mu\text{g-atoms l}^{-1}$. We have made nitrogen measurements on glacial ice recovered from the sea near a green iceberg¹² and on an ice core sample from 66 m below the surface of the Ross Ice Shelf at $82^\circ 22.5'S$, $168^\circ 37.5'W$. The nitrate assay was 3.9 $\mu\text{g-atoms l}^{-1}$ in the iceberg, and 1.1 $\mu\text{g-atoms l}^{-1}$ in the ice core, consistent with the earlier Parker *et al.* data.

Nitrate, phosphate and silicate are the nutrients commonly measured in seawater. These nutrients are present in abundance in the AASW south of the Polar Front and are not generally believed to limit phytoplankton growth. If other compounds such as ammonia ($\text{NH}_4\text{-N}$) are limiting to the rate of primary production⁷, then melting glacial ice may be an important factor, locally. Our ammonia value for the ice shelf core sample ($2.4 \mu\text{g-atoms l}^{-1}$) is similar to the Parker *et al.* mean ($1 \mu\text{g-atoms l}^{-1}$). Both measurements are an order of magnitude greater than surface water ammonia concentrations reported for the Ross Sea ($<0.1\text{--}0.3 \mu\text{g-atoms l}^{-1}$) (ref. 7). The ammonia levels of aliquots drawn from the atypical green iceberg sample ranged from 25 to 33 $\mu\text{g-atoms l}^{-1}$, slightly above the range for South Pole ice core samples⁶. The green iceberg sample also contained quartz grains and metallic particles (J. Anderson, personal communication), fibres, algal cells, and an organic nitrogen level of $241.6 \mu\text{g-atoms l}^{-1}$. A thin section showed it to be derived from a valley glacier (A. Gow, personal communication).

Upwelled deep water is the primary source of nitrate, phosphate and silicate in the AASW. Beneath the AASW, nitrate values are uniformly above 30 $\mu\text{g-atoms l}^{-1}$ (refs 9, 10). This deeper water upwells into the surface layers, replacing waters that mix and sink as Antarctic Bottom Water and Antarctic Intermediate Water. The upwelling rate, based upon temperature, salinity and oxygen-18 budgets, has been estimated to be $60 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ (refs 13, 14), corresponding to a nitrate input to the AASW of $8 \times 10^8 \text{ tonnes yr}^{-1}$. This is four orders of magnitude greater than the meltwater contribution of $3 \times 10^4 \text{ tonnes yr}^{-1}$, derived from the Parker *et al.* mean nitrate for glacial ice and the assumption of an equilibrium ice cap. Nitrate

enrichment of 18% in the euphotic zone of a glaciated Arctic fiord was first attributed to rock flour¹⁵ and later to upwelling¹⁶.

Most iceberg melting occurs below the sea surface in the predominantly above-freezing environment that exists there during all seasons. Holdsworth and others have shown that the amount of downwarping of sea ice attached or frozen to icebergs in d'Iberville Fiord, NWT, Canada, may be accounted for by continuous melting of the submerged portions of the glacier ice throughout the year. Thus, there may be no sharp seasonal glacial ice melting peaks coincident with phytoplankton blooms⁶.

Several associations have been reported between glacial ice and the biological community. Bottom scouring by icebergs affects bottom dwellers in several ways¹⁷. Links have been noted between icebergs or shelf ice and microflora¹⁸, diatoms¹⁹, Arctic terns²⁰, seals in the Arctic¹⁷ and leopard seals in the Antarctic²¹. We have observed Antarctic petrels, snow petrels and Adelie penguins on and in the vicinity of icebergs. Glacial ice contains some 3–8% (by volume) compressed air bubbles²² that may influence mixing processes when the ice melts. The 'sizzling' noise that accompanies the bubble pressure release "should be detectable by sonar . . . out to distances of 50–100 miles against the ambient sea background"²³. If plankton levels are higher near icebergs, then the noise of that melting ice could well attract higher species.

There is evidence that melting glacial ice may influence vertical processes in the surface layers. Neshyba²⁴ has pointed out the potential importance of iceberg-induced upwelling on the oceanography of the Weddell Sea, and Foldvik and Kvinge²⁵ have described the possible thermohaline upwelling of near-freezing water near ice shelves. Few oceanographic observations have been made in the vicinity of Antarctic icebergs. The anomalously high subsurface salinities and temperatures recently reported near a large Antarctic iceberg²⁶ may be an artefact of the instrument or calibration. In the Ross Sea we have observed brash or pack ice surrounding an iceberg (see also Fig. 24 in ref. 27) but separated from it by a ring of open water. This may result from upwelling along the walls, wave and swell interaction, or differential motion between iceberg and smaller pieces of ice with shallower draft. Upwelling was considered responsible for muddy ice-free water at the sea faces of glaciers in Greenland²⁸. Josberger²⁹ reported field measurements of melt-driven upwelling adjacent to an Arctic iceberg, and

modelled both upwelling and downwelling in the laboratory. Several of his vertical temperature–depth profiles suggest additional effects at 50–70 m.

In another laboratory experiment³⁰ meltwater was observed to mix and spread laterally away from ice blocks inserted into salt-stratified water. Huppert and Turner noted the production of a "series of tilted convecting layers which grow out into the environment". They found that "a considerable portion of the melt water can be fed into the environment close to the level at which it is produced and very little, if any, rises to the surface".

We have measured the large scale lateral spreading of water (Fig. 1) that has been cooled by melting at the base of the Ross Ice Shelf³. This Ice Shelf Water³¹ extends northwards to at least the continental shelf break. A similar water mass is found on the continental shelf in the Weddell Sea³² and adjacent to the Amery Ice Shelf³³. Ice Shelf Water temperatures are as low as the *in situ* freezing point; salinity ranges from 34.4 to 34.75‰, but does not usually form a local minimum within the vertical halocline. Ice Shelf Water with potential temperature below -2.0°C has been observed in the Ross Sea at depths from 225 to 525 m. We have hypothesised³ that the main source of heat for melting at the base of the Ross Ice Shelf is provided by an intermediate-depth warmer layer, derived from the Circumpolar Deep Water. Pettersson³⁴ seems to have modelled several of the thermohaline features we observed north of the Ross Ice Shelf.

Similar to flow away from the Ross Ice Shelf (Fig. 1) and ice in the laboratory³⁰, most meltwater from icebergs may spread laterally rather than upwell along the sides. Typical tabular Antarctic icebergs will have initial drafts approximating the depths below sea level of the terminal faces of the ice shelves. A study of 407 Antarctic icebergs³⁵ showed a mean freeboard of 40 m, corresponding to a subsurface draft of 182 m. Mean widths for icebergs observed with Landsat imagery in one Antarctic sector were 0.4 km on the edge of the pack ice and 0.7 km in the pack ice³⁶. For icebergs with dimensions larger than about 0.7 km, basal area will exceed wall area. The base should then be the primary wastage surface³⁴ and this surface will frequently be near the lower levels of the AASW. Alternatively, if calving as a result of wave-induced melting and mechanical erosion were the primary disintegration process³⁷, most melting would take place nearer the sea surface. We have often observed the calving of brash and growler-sized pieces

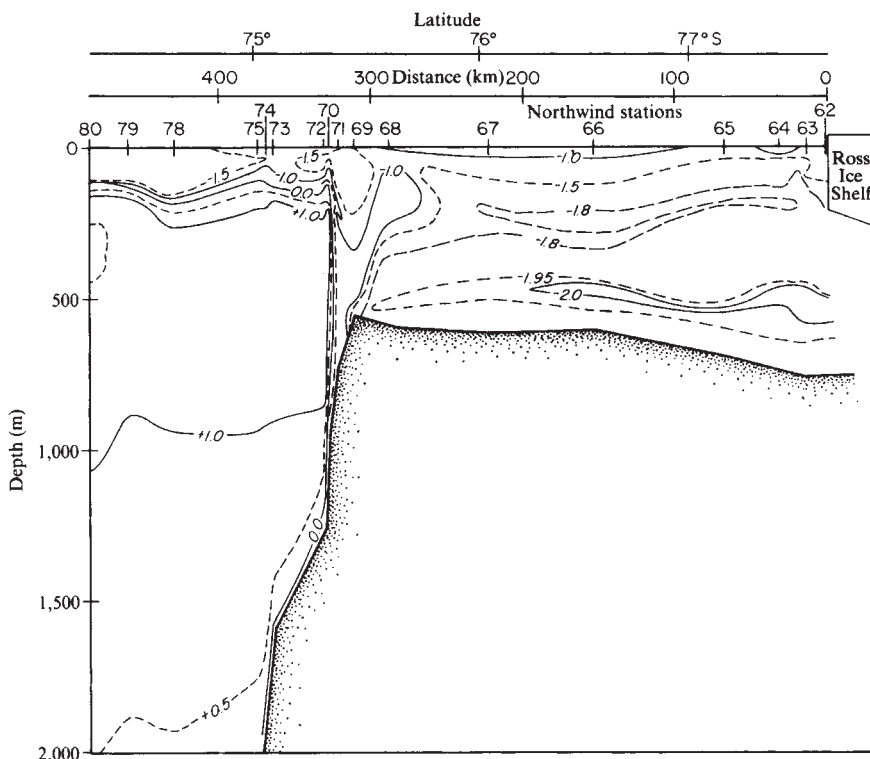


Fig. 1 Potential temperature profile from USCGC Northwind stations taken in December 1976 (ref. 46). The section extends about 500 km north-east of station 62, which was next to the Ross Ice Shelf at 178°E longitude. A layer of ice shelf water³¹ ($\theta < -1.95^{\circ}\text{C}$), that results from melting beneath the ice shelf, reaches the continental shelf break. A shallower layer of ice shelf water ($\theta < -1.8^{\circ}\text{C}$) also extends north from the ice shelf at Antarctic surface water depths. An intermediate depth warmer layer ($\theta > -1.8^{\circ}\text{C}$), better developed on the east-central continental shelf, provides the major heat flux for melting of the glacial ice³.

from the northern face of the Ross Ice Shelf, but note that much of the region populated by Antarctic icebergs and ice shelves is covered by sea ice for most of the year, which should limit wave-induced erosion.

The temperature minimum at the base of the Antarctic Surface Water is generally attributed to vertical winter mixing, with subsequent capping by a warm summer mixed layer³⁸. However, the large latent heat exchange (80 cal g^{-1}) at the base of melting icebergs and subsequent lateral mixing of the meltwater could also contribute to this feature. Where the temperature minimum is shallow, as in the Weddell Gyre, the impact of iceberg melting may extend well below the AASW. The step-structure in the centre of the Weddell Gyre that Huppert and Turner attribute to "systematic injection of meltwater at intermediate depths" is best developed just below the temperature minimum in their Fig. 1b (ref. 30). Over the AASW region south of the Antarctic Convergence, the heat exchanged annually in melting glacial ice should be sufficient to lower by 1°C the temperature of a layer 7.5 m thick. While the upwelling rate used above corresponds to an AASW residence time of only one year, decreasing this rate³⁹ would allow to a greater influence of iceberg melting on the AASW. An order of magnitude more heat will be taken up by the summer melting of pack ice than by the annual melting of glacial ice, but the melting pack ice will primarily involve only the top few metres of seawater. That meltwater will have a stabilising effect on the water column, and the associated latent heat flux will be more than offset by incoming radiation⁴⁰.

The environmental impact of melting icebergs will not be evenly distributed in space and time. Iceberg tracks will be influenced by currents, oceanic fronts, winds, pack ice, bottom topography, and by the location and productivity of drainage areas. From our observations and published accounts^{35,36,42,43} of iceberg distribution and movement, we would expect the maximum impact from melting icebergs to occur in the Antarctic coastal zone. There a cold and fresh water mass, V-shaped in cross-section⁴⁴ is frequently observed over the Antarctic continental slope. It extends to depths of several hundred metres and apparently defines the deeper portion of the surface westwards coastal current⁴⁵. Its low temperature and low salinity^{44,46} probably result in part from melting glacial ice. It may be a source reservoir for both the AASW temperature minimum and the fresh component of Antarctic Bottom Water.

North of the continent, icebergs frequently appear in clusters and are likely to travel preferred routes. The gigantic Trolltunga iceberg that has been tracked by satellite for 11 years (Fig. 23 in ref. 27) may provide evidence for the association of icebergs with convergent frontal zones. Once Trolltunga entered the northern Scotia Sea it seems to have tracked the southern boundary of the Polar Front, possibly grounding as it crossed the North Scotia Ridge. Moving parallel to the bottom contours, it then executed two reversals in an east-west direction, a characteristic feature of the front in that region⁴⁷. To the extent that icebergs are associated with frontal zones⁴⁸, the meltwater may contribute to lower surface salinities sometimes observed there (Fig. 4b in ref. 49).

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Isolation and characterisation of a bipotential haematopoietic cell line

VARIOUS cell lines have been used to study the regulation of proliferation and differentiation, and events occurring during mutagenesis and carcinogenesis. These include 'normal' cell lines which, however, frequently show chromosome changes, especially aneuploidy, and where normality is based essentially on the nontumorigenic capacity of the cells. Whether such cells retain the ability to fulfil their genetically programmed functional state *in vivo* has not been determined. Moreover, although studies using transformed, tumorigenic cells (Friend leukaemia¹, myeloid leukaemia² and neuroblastoma³) have given useful information on some of the factors involved in differentiation, the fact that the cells are tumorigenic indicates that caution must be exercised in the interpretation of results. We report that we have now isolated and cloned an autonomous haematopoietic cell line where the cells have a diploid complement of chromosomes, are non-tumorigenic and bipotential (can be induced to differentiate *in vivo* into two distinct haematopoietic lineages), and which in appropriate circumstances protect mice from potentially lethal irradiation.

The cell line was isolated from a long-term culture⁴ of (C57BL/6 $\times$ DBA/2)F₁ female bone marrow cells, which had been infected with NB-tropic, polycythaemia-inducing Friend

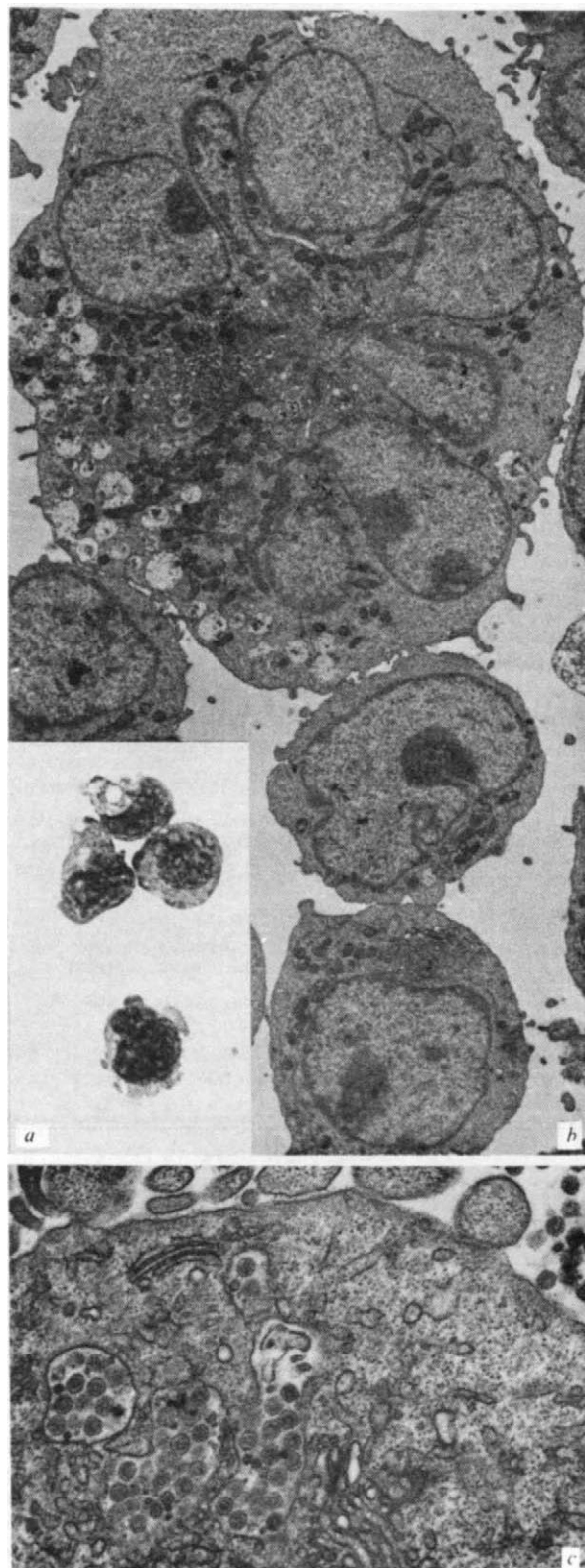


Fig. 1 *a*, Cultured 416B cells stained with May-Grunwald Giemsa. Note large nuclear:cytoplasmic ratio, vacuolated cytoplasm ($\times 750$). *b*, Section through a pellet of 416B cells after glutaraldehyde-osmium fixation, showing the size variation of cultured cells. Three small round cells are apparent (corresponding to *a*), with smooth nuclear profiles and undifferentiated cytoplasmic morphology. The large cell (perhaps a megakaryocyte precursor) is characterised by size, dense mitochondrial population and extensive nuclear segmentation ($\times 4,500$). *c*, Detail of the cytoplasm of an undifferentiated cell, showing vacuoles containing intracellular immature virus particles, lacking the external coat. Extracellular virus particles are also apparent ($\times 26,000$).

leukaemia virus (FLV)⁵. Table 1 shows the essential features of these cultures. For the first 5–6 weeks, the virus-infected cultures behaved similarly to the controls, with both groups demonstrating extensive production of granulocytes (all stages of maturation) and maintenance of haematopoietic stem cells (CFU-S)⁶ and granulocyte precursor cells (CFU-C)^{7,8}. The CFU-S formed spleen colonies containing all haematopoietic elements (erythroid, granulocytic and megakaryocytic).

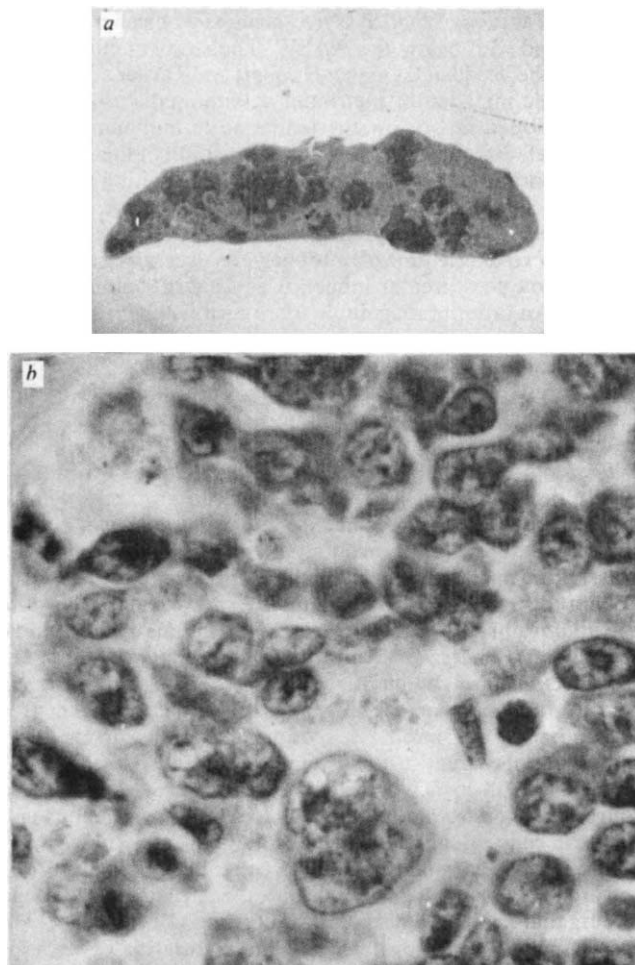


Fig. 2 *a*, Section through mouse spleen (stained with haematoxylin and eosin) showing colonies produced from 416B cells ($\times 5$). *b*, Part of a spleen colony, demonstrating granulocytes in various stages of maturation and a single megakaryocyte ($\times 750$).

Subsequently, haematopoiesis in the control cultures decreased, and eventually only phagocytic mononuclear cells remained. In the virus-infected cultures, on the other hand, there was a sustained granulopoiesis (with a marked shift towards the production of immature forms) and a massive increase in CFU-S. However, as previously reported⁵, such CFU-S show limited differentiation capacity, producing spleen colonies containing only granulocytes and megakaryocytes. No erythropoiesis was observed. It is particularly significant that throughout the culture period, injection of the culture cells or cell-free supernatants into neonatal or adult syngeneic mice did not result in the development of leukaemia at >1 yr post-inoculation. In this respect, therefore, the cultures differ from our previously reported results⁵ where we showed that following infection of genetically susceptible marrow cultures with FLV complex, both the helper (LLV) and the 'defective' (SFFV) viral components replicated, resulting in a maintenance of the erythroleukaemia-inducing capacity of the virus. In the present experiment, although the LLV component is detectable (with weekly titres

Table 1 Characteristics of normal and Friend leukaemia virus-treated long-term marrow cultures

Weeks cultured	Cell count $\times 10^5$	Morphology				Total CFU-S	Total CFU-C
		B	EG	LG	Mono		
1	14.8(11.3)*	4(0)	8(4)	58(62)	30(32)	703(358)	ND(4,380)
5	20.4(54.0)	35(27)	29(30)	23(36)	10(8)	340(1,120)	5,050(18,000)
10	20.8(10.2)	10(3)	70(8)	5(60)	12(28)	1,730(211)	2,670(ND)
14	21.6(6.6)	30(8)	60(10)	1(6)	5(76)	32,940†(0)	ND(328)
16	31.8(1.0)	16(0)	66(0)	1(2)	15(98)	136,740†(0)	ND(0)
18	33.6(3.0)	25(0)	70(0)	0(0)	4(100)	500,000†(0)	ND(0)
20	50.0(1.8)	40(0)	55(0)	0(0)	5(100)	250,000†(0)	>50,000†(ND)

Cultures were established as previously described⁵. The cells were tested weekly for CFU-S and/or CFU-C and smears made for morphological examination. B, Blast cells; EG, promyelocytes and myelocytes; LG, metamyelocytes and polymorphonuclear cells; Mono, phagocytic mononuclear cells; ND, not done.

* Control (normal) values are in parentheses.

† Restricted differentiation capacity.

ranging from 2×10^4 to 8×10^5 plaque-forming units per ml (ref. 9), the erythroleukaemogenic ability (the SFFV component) has been lost. The precise reasons for this are unclear (and now under investigation), but the end result was that we were able to isolate a cell line with unique characteristics.

After 20 weeks of culture (Table 1), aliquots of cells were transferred to fresh culture flasks. After several days, cell proliferation of a nonadherent population was observed and further sub-culture was possible. The cells were designated 416B and samples of these early isolates were cryopreserved. Morphologically, the cells are heterogeneous: the majority are undifferentiated blast cells and 'promyelocytic' cells (Fig. 1a), with occasional larger cells with lobed nuclei. Ultrastructural analysis shows that the majority of cells are undifferentiated, have a large nuclear:cytoplasm ratio, smooth nuclear profiles and limited condensed chromatin (Fig. 1b). A minor proportion of cells shows some nuclear segmentation characteristic of immature granulocytes. The larger multi-lobed cells strongly resemble megakaryocytes (Fig. 1b). Many cells show cytoplasmic and extracellular forms resembling C-type virus particles (Fig. 1c). The population doubling time of the cells is 38–44 h. Karyotype analysis showed that about 95% of the cells possess a diploid chromosome complement, the remaining cells being true tetraploid or octaploid cells; this supports the proposal that the larger cells are developing megakaryocytes.

The colony-forming ability of the cells *in vitro* is shown in Table 2. Cells were plated in the presence or absence of heart conditioned medium¹⁰ as a source of colony-stimulating activity (CSA) which is essential for the growth of normal bone marrow granulocyte precursor cells (CFU-C)¹¹. At low cell concentration, colony formation occurred only in the presence of CSA-containing medium, but at higher cell concentrations, CSA was not necessary. Thus, at high cell densities, the cells apparently produce growth-promoting factors; it is not known whether such factors are equivalent to CSA. The colonies

consisted of loosely dispersed cells, reminiscent of 'macrophage' colonies¹¹, and in this respect differed from the typical compact colonies generally formed by leukaemic murine cells. Morphological analysis of the colony cells showed them to be similar to the parent population, with no obvious differentiation. Individual colonies were isolated from soft agar; all such isolates readily proliferated and produced permanently growing cell lines with characteristics identical to the original parental population, including the cell heterogeneity previously mentioned. We have tried to induce *in vitro* differentiation and maturation of the cells, using a variety of agents that promote differentiation of certain granulocytic or erythroid leukaemia cell lines^{1,2}. These included purified L-cell CSA, purified erythropoietin, dimethyl sulphoxide, 5-bromodeoxyuridine, dexamethasone, and conditioned medium obtained from long-term cultures of normal mouse bone marrow cells. None of these reagents has so far induced morphological differentiation.

The cells do, however, form macroscopically visible colonies in the spleens of irradiated mice (Table 2 and Fig. 2a); although the number produced varied widely, on all occasions the colonies contained only granulocytes and megakaryocytes at all stages of maturation (Fig. 2b). Smears taken from the bone marrow also showed this limited differentiation pattern (Fig. 3a, b). There was no evidence of erythropoiesis or lymphopoiesis. Thus, in the appropriate *in vivo* environment, the cultured cells undergo apparently normal development. However, mice reconstituted with the cultured cells rapidly became anaemic due to defective erythropoiesis, and death occurred 11–20 d post-irradiation. In an attempt to prolong survival, the reconstituted mice were given weekly intraperitoneal injections of 0.5 ml packed red blood cells; 30% of these mice survived for longer than 7 weeks. After 2 weeks only granulocytic and occasional megakaryocytic cells were found. Surface marker analysis failed to demonstrate the presence of Thy-1 antigen or surface immunoglobulin-positive lymphoid cells.

Table 2 *In vitro* and *in vivo* colony formation by 416B cells

<i>In vitro</i>			<i>In vivo</i>			
Cells plated (3 ml)	CSA		Cells injected	Spleen colonies		
	Present	Absent		I	II	III
10^3	15 ± 2	0	5×10^2	20 ± 1	0	0
10^4	182 ± 21	0	10^3	28 ± 3	0	0
10^5	>1,000	>1,000	5×10^3	Confluent	1	0
5×10^5	Confluent colonies	Confluent colonies	10^4	Confluent	2.5	1
			10^5	Confluent	25.3	18.5

In *in vitro* experiments, the cells were plated in 3 ml Fischers medium supplemented with 20% horse serum (Flow) and antibiotics, in 0.3% agar. CSA was added as a 20% mouse heart conditioned medium¹⁰. Results are the mean and standard error of three experiments. In *in vivo* experiments, the cells were injected into mice previously exposed to 800 rads. The animals were killed 8–9 d later and the spleens removed and fixed in Bouin's solution. Individual colonies were counted with a dissecting microscope. Experiments I, II and III were carried out 2, 5 and 10 months, respectively, after establishing the cell line.

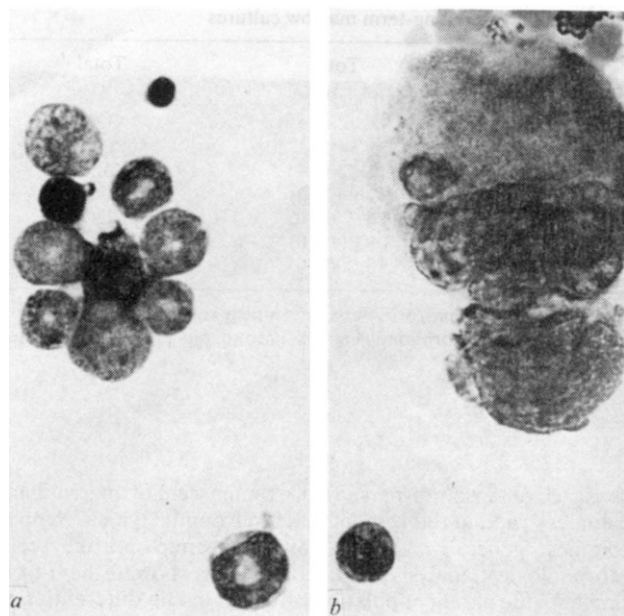


Fig. 3 *a*, Bone marrow taken from mice 2 weeks after injection of 416B cells; note extensive granulopoiesis ($\times 750$). *b*, As *a*, showing megakaryopoiesis ($\times 750$).

At 6 weeks post-reconstitution, spleen and bone marrow cellularity had recovered to near normal values. Within the spleen the granulocytes were the major population, although 14% of the cells were Thy-1-positive lymphocytes; B cells were absent. Granulocytes were also found in the bone marrow and at this time limited erythropoiesis was apparent. Karyotyping of the cells showed that approximately 50% of the spleen cells were of donor origin, whereas in the bone marrow, all the cells were of host origin. This presumably represents re-emergence of a surviving host stem cell population¹² to provide erythropoiesis and T-lymphopoiesis. This finding is not surprising, as the elegant experiments of Micklem *et al.*^{13,14} showed that stem cells differ in their 'competitiveness'. For example, when irradiated mice are reconstituted with a mixture of stem cells derived from the peripheral blood and bone marrow, both populations can initiate haematopoiesis, and presumably both can contribute to the 'short-term' survival of these animals. Within a few weeks, however, the number of 'blood-derived' cells declines and the haematopoietic system is reconstituted with marrow-derived stem cells¹⁴. That system seems analogous to the one reported here and similar mechanisms may be in operation. Indeed, there is increasing evidence to indicate that there is a hierarchy of stem cells (CFU-S) which differ in self-renewal capacity¹²⁻¹⁵ and perhaps in their ability to differentiate. The cell line described here may have arisen from a cell with restricted differentiation potential. The relatively few long-term survivors in our reconstitution system may be related to the delayed onset of lymphopoiesis and consequent severe immunodeficiency in these animals.

Several questions arise. (1) Can the differentiation 'block' be overcome *in vitro*? (2) Is there a sub-population of normal stem cells *in vivo* which has similarly restricted differentiation potential, or is the restriction imposed on the cells following infection with leukaemia virus? (3) Will antisera raised against the cells determine new haematopoietic or differentiation linked antigens? (4) Can the cells be induced to undergo leukaemic transformation and, if so, do specific chromosomal changes¹⁶ occur? The answers to these points will give us valuable information on the nature of haematopoietic cell differentiation and transformation.

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A specific helper factor which enhances the cytotoxic response to a syngeneic tumour

IMMUNE RESPONSES are regulated at least in part by a complex system of cellular interactions which result in the specific enhancement or suppression of the reaction to a given antigen. In many situations soluble 'factors' seem to be able to replace these cells, although whether they actually do so *in vivo* remains unknown. Specific suppressor factors have been demonstrated to influence a variety of humoral and cell-mediated immune responses. Specific helper factors which augment antibody production have been studied extensively¹⁻³, but analogous factors active in cell-mediated immunity have not yet been documented. Soluble factors which enhance the generation of cytotoxicity have been reported by several workers⁴⁻⁸ but none of these has been demonstrated to be antigen specific. We report here the isolation of a helper factor which specifically enhances the generation of cytotoxic cells in a syngeneic mouse tumour system.

We have shown previously that cells from spleen or thymus of mice bearing the P815 mastocytoma, or extracts from these organs, can specifically suppress the generation *in vitro* of cells cytotoxic to P815 (refs 9-11). These results are in basic agreement with those of Fujimoto *et al.*¹² and Green *et al.*¹³, who examined the influence of suppressor cells or factor on the *in vivo* growth of methylcholanthrene-induced tumours. In either assay system the activity measured is a net value which could comprise components of both help and suppression. Fractionation of our crude thymus suppressor factor by isoelectric focusing or by gel filtration indicated that there were fractions showing net enhancement of cytotoxicity suggesting the presence of both a helper and a suppressor factor in the same extract. In subsequent work aimed at the isolation of a helper factor, fractions of spleen extracts separated by gel filtration were used.

Figure 1 shows the effluent profile of extracts from normal or 8-d tumour-bearer spleens separated on G150 Sephadex. The

fractions were added to cultures containing normal DBA/2 spleen cells together with mitomycin-C-treated P815 cells as antigen. After 5 d the cells were collected and tested for cytotoxicity against P815 in an overnight ^{51}Cr -release assay. Results from cultures containing fractions from the tumour bearers were compared with those from cultures containing an equivalent concentration of normal fractions. Only fractions II and III showed significant differences between the tumour and normal extract. Fractions prepared from extracts of spleens taken at various times after tumour injection indicated that suppressive activity, if present, was in fraction II, whereas enhancing activity appeared in fraction III, indicating that the two activities could be at least partially separated on the basis of size (these data will be presented elsewhere). For subsequent work on the purification of a helper factor, fraction III from 8-d tumour-bearer spleens was used.

Anti-P815 cytotoxicity generated in cultures containing tumour-bearer fraction III (TIII) at concentrations between 0.1 and 0.05 spleen equivalents was consistently enhanced (five experiments, data not shown), as compared with controls containing equivalent amounts of normal factor III (NIII). Further purification of the factor was affected by absorbing NIII or TIII on to columns of P815 membrane fragments linked to Sepharose. Bound material was eluted in mildly dissociating conditions with 2 M salt. As shown in Fig. 2a, the eluted material derived from TIII was markedly enhancing at a concentration of 0.05 spleen equivalents. Equivalent results were obtained with 0.005 spleen equivalents, but at lower concentrations than this the helper activity was diluted out (data not shown). However, the control eluate derived from NIII did not influence the response at any concentration tested. The data in Fig. 2a show that the purified helper factor increased the cytotoxicity about fourfold in terms of cytotoxic units. The specificity of the factor was tested by adding it to parallel cultures containing mitomycin-treated L1210 rather than P815 as antigen. Neither fraction had any effect on the generation of anti-L1210 cytotoxicity (Fig. 2b). Similarly, the P815 helper factor did not enhance anti-H-2^d killing generated in cultures of C57BL/6 spleen cells with irradiated DBA/2 spleen cells (data not shown). We have shown previously that cytotoxicity in the syngeneic system is T-cell dependent (and probably T-cell mediated), in that treatment of

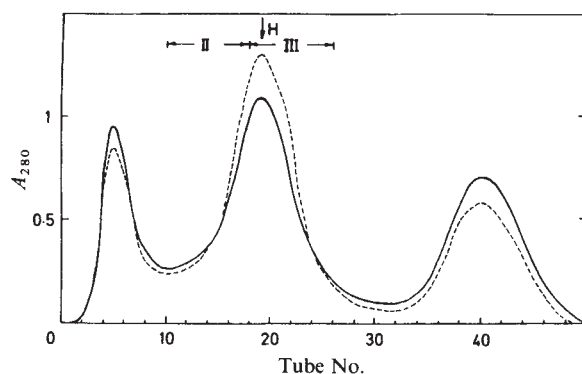


Fig. 1 Fractionation of spleen extracts on Sephadex G150. Extracts were prepared by the technique previously used for thymus tissue¹¹. Spleens were taken from 8–12-week-old normal (broken line) or tumour-bearing (solid line) female DBA/2 mice. The tumour bearers were injected 8 d before death with 2×10^3 P815 mastocytoma cells in the right flank. At the time of death, the tumour was growing as an easily palpable confined mass at the site of injection. There was no evidence of metastasised tumour cells in the spleen by microscopic examination. Five spleens suspended in a small volume of phosphate-buffered saline (PBS) were frozen and thawed, homogenised in a conical glass tissue grinder, sonicated twice for 60 s on ice, centrifuged at 60,000g for 60 min at 4 °C, and the supernatant was immediately applied to the Sephadex column. The column was eluted with borate saline, pH 8.3, collecting 4-ml fractions which were pooled as indicated. Mouse haemoglobin eluted near the peak of the second peak as indicated by arrow H.

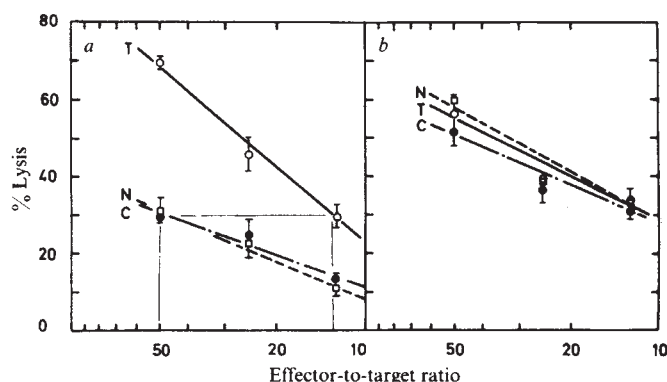


Fig. 2 The effect of P815 membrane column-purified helper factor on the *in vitro* generation of cytotoxicity to syngeneic P815 (a) and L1210 (b) tumour cells. 5×10^6 normal DBA/2 spleen cells were cultured with 5×10^5 mitomycin-C-treated P815 or L1210 cells in 2.5 ml of RPMI 1640 in 24 well Linbro plates (No. 76-033-05). Cultures contained 10% fetal calf serum, 10 mM HEPES, 2×10^{-5} M 2-mercaptoethanol, with or without 0.05 spleen equivalents of test fractions. After incubation for 5 d at 37 °C the cells were collected and set up in a standard 18-h ^{51}Cr -release assay⁹ against ^{51}Cr -labelled P815 or L1210. The results show the averages and standard deviations of triplicate cultures. The P815 membrane Sepharose column was prepared as previously described¹¹ by linking a sonicate of P815 membranes obtained by hypotonic lysis to Sepharose 4B activated with cyanogen bromide. Unreacted groups were blocked with ethanolamine and the absorbent was washed with 0.1 M HCl and 2 M NaCl before use. Fractions were stirred for 15 min at either 4 °C or room temperature with the absorbent. The absorbent was then poured into a small column, washed with PBS until the A_{280} of the eluate was <0.01 and then eluted with 2 column volumes of 2 M NaCl. The A_{280} of the eluted material was in all cases <0.01 . It was stored at -70 °C. Concentration is quoted as spleen equivalents based on the volume of original spleen homogenate, corrected for the change in volume following gel and affinity chromatography. Values for the eluted fractions are maximums based on the assumption that all the activity in the Sephadex fraction was recovered. Identical results were obtained using 0.005 spleen equivalents of purified factor. It can be seen in (a) that for an equivalent level of cytotoxicity, for example, 30%, about a quarter of the number of cells from helper-induced cultures were required as compared to controls, indicating an approximately fourfold increase in cytolytic units. ○, □, Cultures containing eluted fractions derived from TIII or NIII, respectively; ●, control cultures without added factor.

the effector population with anti Thy-1 antiserum and complement before the 18-h abolishes killing¹⁰. This was also true of the enhanced cytotoxicity generated in the presence of P815 helper factor, suggesting that the mechanism of killing was not altered.

Helper factors in other systems^{2,14,15} have been shown to be coded for by the I region of the major histocompatibility complex (MHC). Figure 3 indicates that this is also true for the P815 helper factor. In this experiment, the factor was incubated overnight with the antiserum; any immune complexes as well as residual free mouse immunoglobulin (Ig) were then removed on an anti-mouse Ig immunoadsorbent column. The capacity of the column was sufficient to bind twice the amount in the samples. Thus, free antibody, which might influence the response, was not added to the cultures.

The anti Ia used here was prepared by immunising B10 mice repeatedly with B10D2 splenocytes. To render this serum specific for the Ia region of the H-2^d MHC, it was absorbed exhaustively against P815 cells until it was no longer cytotoxic for these targets in the presence of rabbit complement. The properties of this absorbed antiserum are outlined elsewhere¹⁶. It can be seen that helper activity was absorbed by this antiserum. As controls, either anti H-2^k or normal B10 serum absorbed with P815 (results not shown) were used. The purpose

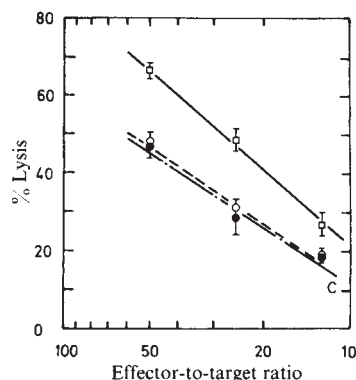


Fig. 3 The effect of anti-Ia antiserum absorption on the activity of P815 membrane column-purified helper factor. The ability of anti-Ia^d to remove helper activity from purified helper factor TIIIe was tested by an immunoadsorbent assay described previously¹¹. Anti-Ia^d antiserum was prepared from B10 anti-B10.D2 antiserum by absorption with P815 as outlined elsewhere¹⁶. The control antiserum was B10.D2 anti-B10.BR. The antiserum and factor were incubated overnight at 4 °C and then passed over an immunoadsorbent column of goat anti-mouse Ig. This material was then added to cultures at a concentration of 0.01 spleen equivalents. Identical results were obtained in a second experiment using P815-absorbed normal B10 serum as the control. □, anti H-2^b; ○, anti Ia^d; ●, control.

of these controls was to show that the loss of helper activity was not due to some inhibitory effect of anti-H-2 antiserum or to the presence of P815 antigen which might combine with the helper factor. In neither case did the control antiserum remove activity. The results of this experiment show that the helper factor bears I-region-coded markers but does not possess constant-region determinants of mouse Ig. They do not exclude the possibility that the factor also has K^d or D^d determinants.

The properties of the P815 helper factor are consistent with those of helper factors active in the antibody response. It binds antigen, is not a conventional antibody molecule, and seems to be I-region coded. Its molecular weight is about 50,000 by gel filtration. Preliminary results from SDS-polyacrylamide gel electrophoresis of iodinated factor in reducing conditions indicate that it is a monomer of MW 65,000. We know of no other reports of antigen-specific helper factors active in the generation of cytotoxic T cells. There is, however, recent evidence of a requirement for specific helper cells in the generation of killer T cells in allogeneic systems.¹⁷ As well as providing an additional way to study the mechanisms that regulate the generation of cytotoxic cells, the factor described here may offer a means of altering the course of tumour growth *in vivo*.

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Cytolytic and proliferative activity of a permanent T killer cell line

ATTEMPTS to establish lymphocyte cell lines with specific cytotoxic activity by viral transformation of cytotoxic thymus-derived lymphocytes (T killer cells) have been unsuccessful in many laboratories. Screening of thymic lymphomas for specific cytolytic activity and attempts to hybridise thymic lymphomas with T killer cells have also been unsuccessful. During these studies it has become apparent, however, that alloreactive T cells derived from a primary mixed lymphocyte culture can be maintained in tissue culture for several months or even years, provided these cells are periodically stimulated with irradiated allogeneic spleen cells^{1,2}. Many of these cell lines, although retaining specific proliferative activity, tend to lose their cytolytic activity after about 6–12 months^{1–4}. This, of course, makes these cell lines less useful for studying the receptor specificity, cytolytic activity or clinical usefulness of T killer cells. Here I report the successful establishment in tissue culture of a cell line which has retained both specific proliferative and cytotoxic activity for almost three years in continuous culture.

Table 1 Cytolytic activity of C · C3 · 11 · 75

	Target*	H-2	Attacker to target ratio	PHA†	% Cytotoxicity‡
5 h					
Expt 1	C3H blasts	kkkkkkk	30:1	–	37 ± 3
	C3H blasts	kkkkkkk	10:1	–	19 ± 1
	R1(TL ⁺)	k . . . k	30:1	–	<1
	R1(TL ⁺)	k . . . k	10:1	–	<1
3 h					
Expt 2	C3H blasts	kkkkkkk	50:1	–	37 ± 2.4
	C3H blasts	kkkkkkk	15:1	–	29 ± 2.2
	BALB/c blasts	ddddd	50:1	–	<1
	BALB/c blasts	ddddd	15:1	–	<1
	C3H blasts	kkkkkkk	50:1	+	43 ± 0.5
	C3H blasts	kkkkkkk	15:1	+	37 ± 0.9
	BALB/c blasts	ddddd	50:1	+	45 ± 0.2
	BALB/c blasts	ddddd	15:1	+	36 ± 0.9
3 h					
Expt 3	C3H blasts	kkkkkkk	50:1	–	40 ± 2.5
	B10A blast	kkkkkkk	50:1	–	43 ± 1.1
	B10A (4R) blasts	kkkkkkk	50:1	–	42 ± 0.8
	ATL blasts	skkkkkd	50:1	–	39 ± 1.1
	C3H OL blasts	ddddd	50:1	–	4 ± 2.7
	BALB/c blasts	ddddd	50:1	–	<1

* Spleen cells (10⁶ per ml) were cultured for 3 d in the presence of 10 μg ml^{−1} bacterial lipopolysaccharide, collected and labelled with ⁵¹Cr (ref. 2).

† PHA concentration was 5 μg ml^{−1} in the assay.

‡ The following equation was used to express cytotoxicity:

$$\% \text{ cytotoxicity} = \frac{(\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})}{(\text{maximal } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})} \times 100$$

Maximal release was calculated by freezing and thawing the target cells three times. Values are means of duplicates. Spontaneous release of targets was as follows: expt 1: C3H = 5.1% per h; R1(TL⁺) = 2.5% per h. Expt 2: C3H = 4.7% per h; BALB/c = 5.2% per h. Expt 3: C3H = 4.8% per h, B10A = 5.1% per h; B10A(4R) = 4.5% per h; ATL = 4.7% per h; C3H OL = 3.4% per h; BALB/c = 5.2% per h.

Mixed lymphocyte cultures containing 10⁵ per ml responder BALB/c spleen cells and 10⁵ per ml 1,000 rad irradiated C3H stimulator cells were set up in Click's minimal essential medium supplemented with 5% selected fetal calf serum, 5 × 10^{−5} M mercaptoethanol, antibiotics and glutamine in 20 ml Falcon no. 3013 tissue culture bottles^{1,2}. Culture flasks were incubated at an upright position in 5% CO₂ in air at 37 °C. Every 2 weeks the

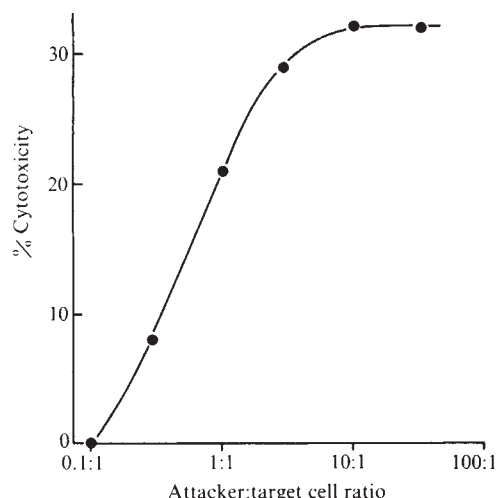


Fig. 1 C·C3·11·75 was titrated for its cytotoxic activity on C3H blast cells at various attacker to target cell ratios. The 3-h time point is given. Spontaneous release of C3H targets was 4.3% per h.

culture supernatant was replaced with new medium containing 10^5 per ml irradiated C3H spleen cells. The culture was regularly split when the cell density reached 5×10^5 cells per ml. The cell culture was called C·C3·11·75 as it is a BALB/c (H-2^d) anti C3H (H-2^k) T killer culture which was originally set up in November 1975 (ref. 2). After 5 months in culture C·C3·11·75 showed strong cell proliferation—about 10-fold over background—when challenged with C3H (H-2^k), CBA (H-2^k) and A (H-2^a) spleen cells but little or no stimulation was caused by DBA/1 (H-2^a), C57BL/6 (H-2^b), ASW (H-2^s) or BALB/c (H-2^d) spleen cells². Strong cytotoxic activity was assayed on H-2^k spleen blast cells or tumour cells². Beginning at about 10 months the cytolytic activity of C·C3·11·75 as assayed on tumour targets gradually disappeared as previously seen with other T killer cell lines¹⁻³. However, as shown in Table 1, expt 1, cytolytic activity to C3H spleen blast cells is retained, while R1(TL⁺) which expresses both the K end private specificity H-2.23 and the D end private specificity H-2.32 (ref. 5) is not lysed. Syngeneic BALB/c spleen blast cells are only lysed in the presence of phytohaemagglutinin (PHA) which only slightly increases cytotoxicity on C3H spleen cell blasts (Table 1, expt 2). The failure of C·C3·11·75 to lyse R1(TL⁺) suggested that this killer cell line recognises other than the K or D region coded antigens. In support of this, Table 1 expt 3 shows that not only C3H, but also B10A, B10A (4R) and ATL, spleen blast cells are lysed. In contrast, C3H OL and BALB/c blast cells are not lysed. Taking the failure of C·C3·11·75 to lyse R1(TL⁺) into account this would suggest that the determinants recognised by this cell line are encoded by genes to the right of the K region, that is, in the IA subregion⁶. As genes in this region are responsible for T-cell proliferation it was tested whether the spleen cells which are susceptible to lysis by C·C3·11·75 are able to stimulate cell proliferation. It can be seen from Table 1 that C3H OL does not stimulate cell proliferation, whereas BALB/c shows consistently significant stimulation. It is not clear what the basis of this stimulation might be. Cloning of this cell line will resolve the question of whether this cell line contains alloreactive as well as autoreactive cells. Aside from the stimulation by BALB/c much higher stimulation is seen with C3H, B10A and ATL spleen cells, which are good targets in the cytotoxic reaction. It therefore seems that the original population of killer cells was heterogeneous, consisting of cells which recognise several specificities expressed on both tumour cells and spleen blast cells. Prolonged selection of this cell line by periodic stimulation with C3H spleen cells has resulted in a population which only kills targets expressing antigens coded by the IA subregion genes⁶. It is possible but not certain that these

same antigens are responsible for the proliferation of this cell line. C·C3·11·75 is sensitive to anti- θ and complement lysis and is also lysed by an anti H-2^d serum in the presence of complement. It can be frozen in liquid nitrogen and will start to proliferate after thawing with a doubling time of ~45 h. Both its proliferation and cytolytic activity depend on weekly stimulation with C3H spleen cells. A titration curve of its cytotoxic activity is shown in Fig. 1. Interestingly, the cytotoxicity has reached plateau values at attacker to target ratios lower than 10:1. This supports the contention that C·C3·11·75 is a rather homogeneous T killer cell population. Maximal cytotoxicity is always found to be in the range 30–45% which may be because of heterogeneity in the target population or inhibition of the effector cells by soluble antigen.

Table 2 Proliferative activity of C·C3·11·75

Responder*	Stimulator	H-2	Proliferative activity† (c.p.m.)
5×10^4 C·C3·11·75	—	—	68 ± 13
—	BALB/c	ddddddd	77 ± 4
—	C3H OL	dddckkk	115 ± 15
—	C3H	kkkkkkk	100 ± 3
—	B10A	kkkddd	77 ± 7
—	ATL	skkkkkd	76 ± 3
5×10^4 C·C3·11·75	BALB/c	ddddddd	$1,590 \pm 192$
5×10^4 C·C3·11·75	C3H OL	dddckkk	168 ± 16
5×10^4 C·C3·11·75	C3H	kkkkkkk	$12,138 \pm 2,057$
5×10^4 C·C3·11·75	B10A	kkkddd	$8,514 \pm 723$
5×10^4 C·C3·11·75	ATL	skkkkkd	$5,802 \pm 118$
5×10^4 C·C3·11·75	Con A	—	—
	supernatant‡	—	137 ± 22

* 5×10^4 C·C3·11·75 were cultured with 5×10^6 per ml 1,000-rad irradiated stimulator spleen cells in 1-ml tubes in RPMI-1640 medium (ref. 2).

† Cultures were labelled with $0.5 \mu\text{l}$ ^3H -thymidine on day 2 and collected on day 3 (ref. 2).

‡ Con A supernatant was prepared by culturing mouse spleen cells with Con A for 2 days. The supernatant was filtered and tested as described in refs 7 and 8.

Recently it was reported that alloreactive killer cells can be propagated up to 5–7 months by weekly stimulation with supernatants of spleen cells stimulated with concanavalin A (Con A)^{7,8}. It is likely that these cells which can be stimulated by Con A supernatant are quite different from those described here⁸. One important difference is that C·C3·11·75 cannot be stimulated by Con A supernatants (Table 2) but by spleen cells while the converse is true for the killer cells described by Nabholz *et al.*⁸. The establishment of an apparently continuously proliferating cytotoxic T-cell line which has retained specific biological functions may be the ideal tool to study the cell surface receptors and the function of this important class of lymphocytes.

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An Lyt differentiated thymocyte subpopulation detected by flow microfluorometry

DATA on the T-cell antigens, Lyt 1, 2, 3 (refs 1, 2), relating to functional T-cell subsets, suggest that some peripheral T cells express restricted Lyt phenotypes, that is, Lyt 1⁺23⁻ or Lyt 1⁺23⁺ (ref. 3). Evidence has been presented showing that two major subpopulations of lymphocytes, Lyt 1⁺ T cells and Lyt 23⁺ T cells, are committed to particular lines of functional differentiation in the immune response before encountering exogenous antigen in peripheral lymphoid tissue^{4,5}. It has been suggested that the two major subpopulations representing T-cell help (Lyt 1⁺) and T-cell cytotoxicity or suppression (Lyt 23⁺) arise in the periphery from peripheral Lyt 123⁺ cells⁶. Recent studies have concluded that as stem cells mature in the thymus, the thymic epithelium is important in determining which H-2 specificities the maturing T cells are able to recognise as self⁷. Previous cytotoxicity data have been interpreted as indicating that Lyt 1, 2, 3 antigens are expressed on each of most if not all, mouse thymocytes; that is, that most thymocytes are Lyt 123⁺ (refs 1-4, 8-10). However, the data presented here suggest that the commitment to differentiation of the Lyt 1⁺ or the Lyt 123⁺ phenotype, and thus presumably the relevant functional differentiation occurs before cell migration from the thymus. Using flow microfluorometry (FMF) and anti-Lyt antisera, we demonstrate a normal thymocyte subpopulation which is not expressing Lyt 2 or Lyt 3 antigens (Lyt 1⁺23⁻). This subpopulation represents a significant constant proportion (10%) of

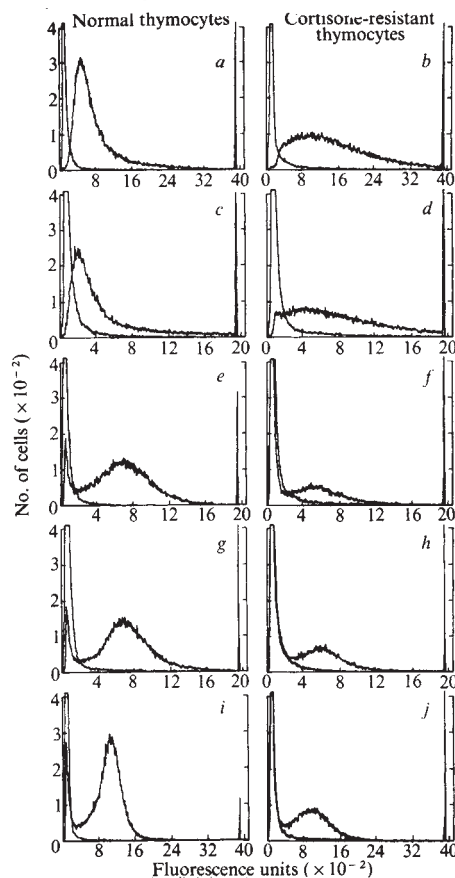
normal thymocytes which is markedly increased following *in vivo* cortisone treatment. The data also show that Lyt 1 expression, rather than being decreased on cortisone-resistant thymocytes (CRT), as would have been predicted from previous cytotoxicity data^{2,8}, is expressed in increased amounts on CRT.

We have used thymocytes of C57BL/6 (B6) mice or the appropriate B6/Ly congenic strains, anti-Lyt antisera and affinity-purified anti-mouse immunoglobulin (Ig) fluorescent staining reagents to re-examine the expression of Lyt 1, 2, 3 antigens on the thymocyte surface. Preparation of these sera and reagents has been described in detail elsewhere¹¹⁻¹³. Immunofluorescence was analysed by FMF¹⁴ on a FACS II interfaced to a PDP 11/40 computer¹⁵. This allowed us to quantitate total cell surface immunofluorescence on individual cells.

Figure 1a-d indicates that both anti-Lyt 1.1 and anti-Lyt 1.2 react with >99% of all thymocytes. The Lyt 1 expression for both alleles is increased on CRT (Fig. 1b, d) relative to normal thymocytes (Fig. 1a, c), as indicated by the increased level of fluorescence on Lyt 1⁺ cells; for example, 50% of CRT have >1,200 fluorescent units per cell compared with 19% of normal thymocytes (Fig. 1a, b). In contrast, anti-Lyt 2.1 and anti-Lyt 2.2 (Fig. 1e-h) react with only 90% of normal thymocytes and either allele is expressed on only 30-50% of CRT. When the Lyt 2 reagents were used at saturation, the mean peak of fluorescence of the positive cells on the cell frequency distribution histogram indicated that Lyt 2⁺ CRT cells were expressing less detectable antigen on the surface than normal thymocytes. Normal B6 thymocytes from neonates are similar to those from adults in Lyt expression, indicating a constant 10% Lyt 2⁻ thymic subpopulation at least from birth.

There is an apparent correlation between the expression of Lyt 3 and that of Lyt 2. (1) The same number of normal

Fig. 1 Normal or cortisone-resistant thymocytes from 3-5-month C57BL/6 (B6) or appropriate B6/Ly congenic mice were treated with anti-Lyt antisera and fluorescent goat anti-mouse immunoglobulin (FI-GAMlg). CRT were obtained 36-40 h after intraperitoneal administration of 0.25 mg cortisone acetate in suspension. For each analysis, 10⁶ cells, suspended in phenol red-free HBSS modified with 0.1% NaN₃ and 0.1% bovine serum albumin were incubated for 45 min at 4°C with antiserum and washed twice by centrifugation in 2 ml volumes of the same medium. The anti-Lyt-sensitized cell pellets were then resuspended in about 50 µl residual liquid, incubated for 30 min with FI-GAMlg, washed twice, resuspended in 0.4 ml of modified HBSS, and analysed by FMF on a FACS II (Becton Dickinson) which is equipped with an argon ion laser operated at 488 nm wavelength and 200 mW for fluorescein excitation. The beam is focused by a 39-mm focal length spherical lens. Fluorescence is detected with an S-11 response photomultiplier tube at 520 V with 520-nm and 530-nm barrier filters. Each section of the Figure displays two cell frequency histograms, positive thymocytes (normal or CRT) and the appropriate negative control cells (normal or CRT), treated equally with the same antiserum and fluorescent reagent. Each histogram represents the fluorescent staining intensities of 50,000 viable cells; viability determined by light scatter¹⁴. Data were collected on the FACS II in a 1,000 channel distribution and stored in a PDP 11/40 computer (Digital Equipment Corporation) interfaced to the FACS II (ref. 15). Fluorescence unit values have been corrected for differing amounts of amplification used when collecting fluorescence signals from cells treated with different reagents. a, b, Anti-Lyt 1.1; positive thymocytes and CRT were obtained from B6/Ly 1.1 mice, control cells were obtained from B6 mice. Cell suspensions were incubated with 10 µl of anti-Lyt 1.1 obtained from a pool of multiple bleedings from a single mouse. This pool and ~20 similar pools were obtained from (B6/N × BALB/c AnN)F₁ mice immunised 3-10 times with B6/Ly 1.1 normal thymocytes. All antisera were routinely absorbed with lymphoid cells expressing the opposite Lyt allele, and were centrifuged at 100,000 g (30 p.s.i., in a Beckman airfuge) for 20 min to remove complexes and aggregates immediately before use. c, d, Anti-Lyt 1.2; these cell suspensions were the same as those in a, b, except that this antiserum recognises the opposite Lyt 1 allele and therefore B6 cells are the positive populations, and B6/Ly 1.1 cells are negative. Anti-Lyt 1.2 was obtained from a pool of C3H/An mice immunised with CE normal thymocytes. 25 µl of this antiserum was used for analysis of Lyt 1.2 expression. e, f, Anti-Lyt 2.1; the positive cell populations in this case were obtained from B6/Ly 2.1, 3.1 mice, and cells from B6/Ly 1.1 mice were used as controls. This antiserum was raised by immunisation of B6/H-2^k mice with CE normal thymocytes. The antiserum was from a highly cytotoxic pool and was also extremely high in anti-Lyt activity measured by this method only 10 µl at 1/10 being needed for these analyses. g, h, Anti-Lyt 2.2; the positive cell populations were obtained from B6 mice and the negative cell populations from B6/Ly 2.1, 3.1 mice. Anti-Lyt 2.2 anti-serum was obtained as described for the anti-Lyt 1.1 except that (C3H/HeN × B6/Ly 2.1)F₁ mice were immunised with normal B6 thymocytes. This antiserum was used at 15 µl for saturation level of staining analyses. i, j, Anti-Lyt 3.2; positive cell populations were obtained from B6/Ly 2.1 mice and negative cell populations from B6/Ly 2.1, 3.1 mice. Anti-Lyt 3.2 antiserum was made from a pool of immunised mouse sera selected for high levels of cytotoxicity. The mice used for this serum were C58 immunised with CE normal thymocytes. Saturation levels of this antiserum were obtained with 15 µl of 1/10 diluted antiserum.



thymocytes are positive with reagents detecting either antigen (compare Fig. 1 *e.g.i*); (2) like Lyt 2 expression on CRT, a higher proportion of Lyt 3⁺ cells is seen with CRT than with normal thymocytes (compare Fig. 1*i, j*); (3) treatment with both anti-Lyt 2 and anti-Lyt 3 still leaves 10% normal thymocytes unstained.

Each anti-Lyt 2 and anti-Lyt 3 histogram shows two nearly symmetrical peaks (Fig. 1*e-j*) one of which falls beneath the negative-control histogram peak and contains about 10% of the total cells, the second of which falls almost entirely outside the range of the negative cell control and contains 90% of the total cells. If, in non-saturating conditions, increasing amounts of anti-Lyt 2 are added, the entire dominant positively stained peak of cells is shifted to the right (brighter), whereas the minor peak of negative cells (unstained cells) is unchanged. By contrast, each histogram obtained with anti-Lyt 1.1 or anti-Lyt 1.2 shows one major asymmetrical peak containing >99% of the cells. Although this peak partially overlaps that of the negative control, several lines of evidence indicate that all cells under the major peak are Lyt 1⁺: (1) if more anti-Lyt 1 is used, the entire major peak shifts to the right (brighter), such that it no longer overlaps the control peak; (2) the major peak is always unimodal with a trail of bright cells, and (3) a small distinct peak (<0.5% of cells) invariably precedes the major peak and may represent Lyt 1⁻ thymocytes. In summary, the frequency distribution histograms for normal thymocyte expression of Lyt 1 can be described as a major peak of 'dull' positive cells with a trail of 'bright' cells. In contrast, the Lyt 2.3 histograms can be described as a minor peak of negative cells and a major peak of positive cells with relatively homogeneous staining. The CRT pattern for Lyt 1 is a relatively flat distribution of positive cells with two approximately equal peaks of Lyt 2⁺3⁺ and Lyt 2⁻3⁻ cells. Thus, the patterns of Lyt 1.1 and Lyt 1.2 antigen expression resemble each other, as do those of Lyt 2.1, Lyt 2.2 and Lyt 3.2. Expression of Lyt 1 antigen is, however, different from Lyt 2 and Lyt 3.

Dual-parameter analysis of forward light scatter (1–15°), a relative measure of thymocyte size¹⁶, and of immunofluorescence for expression of these Lyt antigens was carried out on normal thymocytes and CRT. These analyses indicate that Lyt 23⁻ cells are medium to large in size and express relatively high amounts of Lyt 1. In contrast, the majority of Lyt 23⁺ cells are smaller and express lower amounts of Lyt 1. Figure 2 shows light scatter profiles of B6 CRT showing different staining levels. It is important to remember that fluorescence intensity by this method reflects total antigen expression, not necessarily antigen density.

Recently we described a series of mouse thymic lymphomas which seemed to display limited or restricted Lyt phenotypes similar to functional differentiated, peripheral T cells¹³. Most of the tumours in that study were TL⁺. As TL antigen expression is restricted to thymocytes and T-cell lymphomas¹⁷, we suggested that a normal thymocyte subpopulation existed which was TL⁺ and already differentiated for Lyt antigen phenotype. Other data¹⁸ also predict the functional differentiation of TL⁺ thymocytes. The results presented here confirm the existence of a significant subpopulation of normal thymocytes which are differentiated in expression of Lyt antigens. Similar results have been obtained for several other strains of mice, including BALB/c, A/J, C3H and DBA/2.

The increased expression of Lyt 1 on CRT, as detected by these antisera and FMF, was not predicted by previous cytotoxicity data^{4,8}. Increased expression of Lyt 1 cannot be attributed to induction of antigen expression during the tests because the entire procedure was carried out at 4°C in Hanks' balanced salt solution (HBSS) containing 0.1% NaN₃.

The increased expression of Lyt 1 on CRT is probably not attributable to Fc binding of the anti-Lyt antisera because; (1) there are marked fluorescence profile differences between anti-Lyt 1 and anti-Lyt 23 on the same cell suspension (2) appropriate negative congenic cell controls are not stained in the same conditions with the same antisera (see Fig. 1). (3) staining a

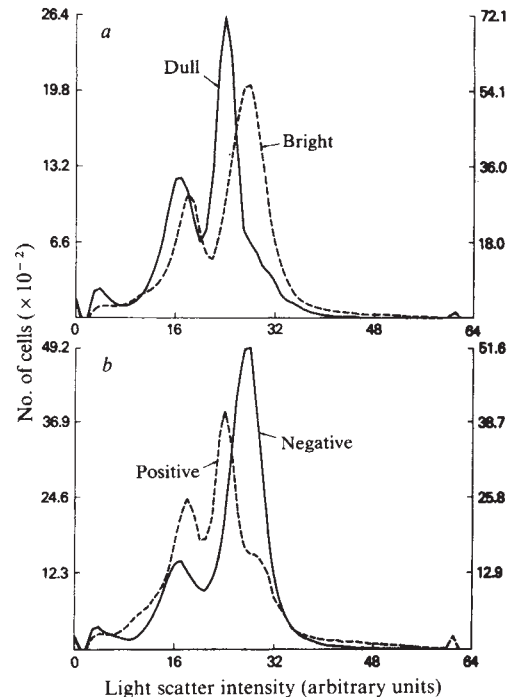


Fig. 2 CRT from C57BL/6 (B6) mice were prepared and treated with anti-Lyt antisera and FI-GAMlg as described for Fig. 1. FMF analysis was carried out as described in Fig. 1, except that data were collected and stored in the computer on both light scatter (LS) and fluorescence (FI) simultaneously for 10^5 total cells (not selected on LS). Data were collected in a 64×64 matrix such that the x-axis represented LS, the y-axis FI, and the z-axis the cell number at each intersection of LS and FI. Data collection and analysis are described elsewhere¹⁵. Here, upper and lower thresholds (windows) were selected on FI intensity and all LS data within each window are shown as the number of cells versus increasing LS. *a*, The LS of B6 CRT staining with different intensities of anti-Lyt 1.2. Dull cells (—) were selected as those with intensities of <384 FI units (see Fig. 1*d*). This corresponds to the majority of normal thymocytes and 20.6% of viable CRT in this example. The bright (---) cells were selected as those with intensities >640 FI units (see Fig. 1*d*) and here represented 65% of the viable CRT. The first peak (cells <21 LS units) in all the histograms in this Figure represents dead cells which have lower scatter intensity than viable cells¹⁴, and which in these CRT preparations comprised about 30% of the total cell population. *b* The LS of B6 CRT staining with different intensities of anti-Lyt 2.2. the negative (—) cells were selected as those with intensities <150 FI units (Fig. 1*h*) and represented 51.5% of the viable CRT in this experiment. The positive cells (---) were selected as those with intensities >260 FI units (Fig. 1*h*) and represented 44.8% of the viable CRT.

50:50 mixture of cells from mice expressing both alleles (for example, Lyt 1.1 plus Lyt 1.2) yields only 50% positively stained cells (data not shown). This considerably decreases the possibility of activation of Fc regions of Ig leading to nonspecific staining of Lyt 1⁻ cells. (4) As a precaution, all alloantisera and fluorescent reagents were ultracentrifuged at 100,000 g before being used in these tests.

The discrepancy between complement-mediated, antibody-dependent cytotoxicity and FMF analysis may be explained by one or more of the following. (1) Our technique is more accurate in terms of the ability to measure the quantity of surface antigen expressed on individual cells. Thus, we were able to detect the minor Lyt 1⁺, 23⁻ subpopulation of thymocytes. (2) There might be a quantitative difference in the expression of Lyt 1, and cytotoxicity assays with complement might kill only Lyt 1 'bright' cells. Varying levels of Lyt 1.1 antigen expression on peripheral T cells have already been suggested to explain a titrated depletion of cytotoxic effector T cells with anti-Lyt 1.1¹⁹. However, other factors are involved, because easily killed normal thymocytes express less Lyt 1 than do CRT by quantitative immunofluorescence (Fig. 1*a-d*). Furthermore, there is no selective loss of Lyt 1 bright cells after cytotoxic depletion of CRT (data not shown). (3) Complement-mediated, antibody-dependent cytotoxicity only detects surface antigen expression when the antigen is recognised by antibody capable of binding

complement, for example, antibody of the IgG2 or IgM classes. Most of the presently available highly cytotoxic pools of anti-Lyt antisera contain considerable amounts of non-cytotoxic IgG1, anti-Lyt antibody (manuscript in preparation), which may block effective complement-mediated cytotoxicity. Using a selected pool of anti-Lyt 1.2, with no detectable IgG1, we have been able to kill >90% of CRT. (4) Other cell surface changes related to the differentiated state of the CRT may make cells more or less sensitive to antiserum plus complement. Recent work has suggested that specific lipids inserted into the membranes of tumour cells reduce the sensitivity to such cytotoxicity²⁰. (5) The genetic locus for Lyt 1 may be a complex with more than one specificity. For example, a specificity, Lyt 1.1^a, may define a T^H subset capable of helper cell function, whereas Lyt 1.1^b defines a T-cell subset required for delayed-type hypersensitivity. These specificities could potentially be further defined by genetic differences, or the use of monoclonal antibodies obtained by anti-Lyt-myeloma fusions. Extensive analysis of the Lyt 1 alleles has been complicated by the fact that the titre of the anti-Lyt 1.2 antisera is much lower than that of anti-Lyt 1.1 antisera. This has also hindered comparative analysis of these antigens from previous cytotoxic data^{19,21}. However, results with a limited number of anti-Lyt 1.2 antiserum pools indicate that expression of Lyt 1.2 closely resembles that of Lyt 1.1 in the FMF immunofluorescence analysis of thymocytes, CRT and peripheral T cells. Furthermore, preliminary evidence suggests that peripheral T cells display similar Lyt 123 phenotypes to those detailed here for CRT.

The accepted hypothesis suggests that all thymocytes and therefore all peripheral T cells express Lyt 123 during differentiation³. Our data suggest that there may be two lines of thymic differentiation; one line which expresses Lyt 1 but not Lyt 2 or 3 and a second line which expresses all three antigens during differentiation. Thus, there would be two thymocyte sublines corresponding to Lyt 1⁺ cells and Lyt 123⁺ cells. The functional capabilities of the peripheral T cells could be determined by or reflect the phenotype expressed in the thymus.

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Substance P causes direct depolarisation of neurones of guinea pig interpeduncular nucleus *in vitro*

SEVERAL lines of evidence suggest that the undecapeptide, substance P, is involved in synaptic transmission in various areas of the central nervous system¹. The development of a specific radioimmunoassay and new immunohistochemical techniques has allowed the identification of several specific but putative pathways involving substance P in the brain^{2,3}. In particular, the cell bodies in the medial habenula and probable nerve terminals in the interpeduncular nucleus contain high concentration of substance P³. In addition, lesioning of the habenula causes a drastic decrease in the substance P concentration in the interpeduncular nucleus⁴. These findings imply that substance P may act as a transmitter in the habenulo-interpeduncular system. Therefore, in the present study, the effect of substance P has been examined *in vitro* on the interpeduncular neurones in an isolated guinea pig brain stem preparation. Substance P depolarised the membrane of the interpeduncular neurones. This finding supports the possible transmitter role of substance P in the central nervous system.

The brain of an adult guinea pig of either sex was removed from the skull, placed on filter paper soaked with Krebs solution with the ventral surface of the brain facing upwards and divided along the midline. A block of the brain stem which included the thalamus, hypothalamus, mamillary body and interpeduncular nucleus was made by removing the majority of the brainstem, hippocampus and the cerebral cortex. A slice ~500-600 µm thick was cut from the medial surface using a stainless steel slicer. Incubation, stimulation and recording procedures are described in detail elsewhere⁵. The slice was continuously perfused with Krebs solution equilibrated with 97% O₂ and 3% CO₂ at 36°C. Under a binocular microscope the stimulating electrodes, consisting of a pair of tungsten wires were placed gently on the habenulo-interpeduncular tract. For extracellular and intracellular single cell recordings, conventional glass microelectrodes were used.

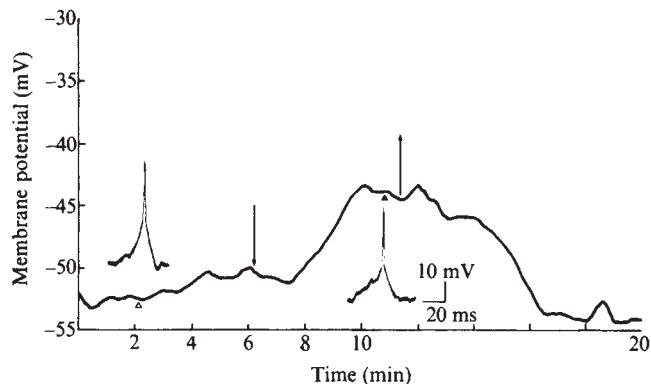


Fig. 1 Effects of substance P on the resting membrane potential of the interpeduncular neurone. Upward and downward arrows represent the duration of a perfusion with a Krebs solution containing 10^{-7} M substance P and the commencement of washing with normal Krebs solution. Inserts illustrate action potentials recorded at points indicated by (Δ) and (▲). Upward deflections represent positive polarity.

Intracellular recordings from interpeduncular neurones show their resting membrane potential to be 52.8 ± 6.7 mV. Each neurone responded to stimulation of the habenulo-interpeduncular tract (50–100 μ s duration) with an excitatory post-synaptic potential which evoked an action potential. No hyperpolarising potentials were observed. In most records spontaneous firing occurred.

In 10 stable extracellular records, substance P (10^{-7} M) was shown to increase reversibly the firing rate of the interpeduncular neurones 1.5 to 5-fold except for one neurone on which no obvious change occurred. L-glutamate also increased the firing rate of the interpeduncular neurones and dose-response curves show 10^{-5} M L-glutamate to be roughly equipotent with 10^{-7} M substance P. In Fig. 1, intracellular records show that substance P (10^{-7} M) induced a depolarisation of ~ 8 mV. The original membrane potential was restored by washing. The excitatory action of substance P was preserved in low calcium medium.

Although the excitatory action of substance P observed in the present study may be nonspecific, comparable studies on slices cut from the hippocampus and cerebellar cortex which contain almost no substance P show substance P to exert no effect. Similar negative results were reported by Dodd and Kelly⁶.

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Depolarisation-induced changes in muscarinic cholinergic receptors in synaptosomes

PROLONGED exposure of receptors to ligands can result in a reduction in the number of available binding sites^{1–8}. This constitutes one possible homeostatic mechanism in the control of interneural communication as there is a functional desensitisation of target tissues. The association between transmission-linked events and the extent of ligand binding can be investigated conveniently in synaptosome preparations. Here we present evidence for what may be a similar desensitisation phenomenon for pre- or postsynaptic muscarinic acetylcholine receptors on synaptosomal membranes as a result of depolarisation mediated via prolonged or persistent opening of sodium channels.

Depolarisation of synaptosomes and cells by electrical stimulation or veratrine treatment cause, through membrane depolarisation, augmentation of metabolic rates, Ca^{2+} uptake, membrane protein phosphorylation, Ca^{2+} dependent transmitter release, cyclic GMP levels, and increased turnover of neurotransmitters^{9–13}. All these effects, together with K^{+} efflux, are abolished or greatly reduced by blocking the Na^{+} channels with tetrodotoxin, which, in the absence of stimulation, has no observable effect^{9,10,12,13}.

Muscarinic receptors in the rat brain have been characterised in detail using tritiated agonists and antagonists^{14–18}. The binding of one such radiolabelled antagonist, [^3H]N-methylatropine

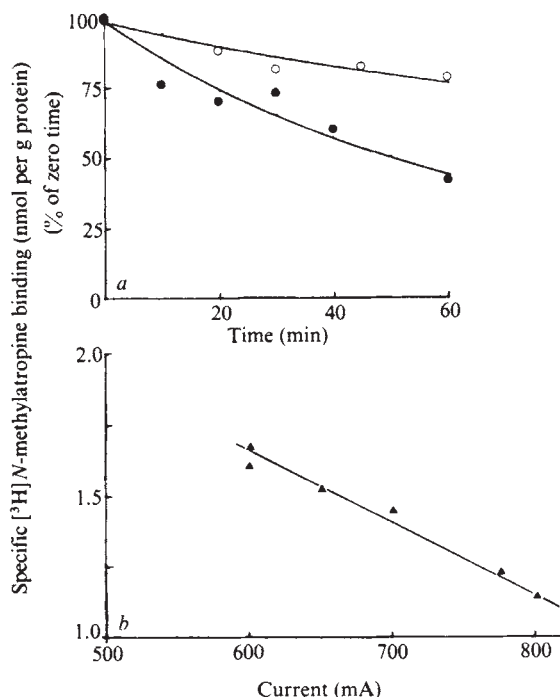


Fig. 1 Time and stimulus-dependence of synaptosomal receptor population. Synaptosomes were prepared from cerebral cortex¹⁹ and incubated at 37 °C in Krebs-phosphate medium containing 10 mM glucose in Warburg respirometer flasks. *a*, Stimulation was applied through gold-ring electrodes for the times indicated, and the reaction was terminated by centrifugation. Hypo-osmotic lysis of the resultant pellets, at 0 °C in 10 mM phosphate buffer pH 7.0 followed by centrifugation of the lysed suspension gave a fraction of synaptosomal membranes. These were stored at -80 °C before measurement of receptor binding capacity as described in Table 1 legend. The open symbols and closed symbols represent binding capacities in the absence and presence respectively of electrical stimulation. *b*, The relationship between receptor loss and the size of the stimulus was investigated by changing the current delivered through the electrodes. In each case, stimulation lasted for 30 min. A typical experiment is shown for a group of six samples each of which showed a different rate of decline of antagonist binding to the muscarinic receptors.

(^3H -NMA), to muscarinic receptors on synaptosomal membranes was greatly reduced (up to 50%) following treatment of synaptosome preparations¹⁹ with electrical pulses, and to a lesser extent, after veratrine treatment. This effect was totally abolished when tetrodotoxin (1 μ M) was present during stimulation (Table 1). With increasing duration of stimulation, the number of receptors decreased at a considerably faster rate than did the control unstimulated, synaptosome membranes (Fig. 1a). In addition, the extent of the reduction in ligand binding appeared to be proportional to stimulus size over the current range 600–800 mA (Fig. 1b). Depolarisation with 56 mM K^{+} in contrast produced no change in the number of antagonist binding sites.

It may be that the depolarisation-induced loss of ^3H -antagonist binding is due to the action of cytoplasmic or lysosomal enzymes (or other soluble factors) released from synaptosomes. These could modify the ligand-binding properties of receptors by proteolysis or other biochemical actions. This possibility was investigated in several ways. The presence of chymotrypsin (3 μ M) during the 1-h synaptosome incubation did not cause any reduction in binding nor did inclusion of phenylmethyl sulphonyl fluoride (a serine protease inhibitor) at 30 μ M prevent the electrically stimulated loss. The effects of endogenous synaptosomal proteolytic enzymes on muscarinic receptors were tested by adding synaptosomal lysates to intact synaptosome preparations. Addition of freeze-dried protein

Table 1 Effect of depolarising agents and neurotransmitters on subsequent muscarinic antagonist binding to synaptosomal membranes

	³ H-antagonist bound per g synaptosomal protein (% of control values)									
	Electrical pulses		Veratrine		K ⁺		Glutamate		Carbachol	
Control	100	(8)	100	(9)	100	(6)	100	(4)	100	(8)
Tetrodotoxin	95 ± 2	(3)	98	(2)	—	—	—	—	—	—
Stimulation	58 ± 5*	(15)	83 ± 2	(9)*	106 ± 4	(9)	107 ± 5	(6)	105 ± 4	(17)
Stimulation in presence of tetrodotoxin	93 ± 1	(4)	97 ± 4	(5)	—	—	—	—	—	—

Synaptosomes prepared from rat cerebral cortex¹⁹ were incubated at 37 °C in Krebs-phosphate medium containing 10 mM glucose and tetrodotoxin (1 µM), where appropriate, in Warburg respirometer flasks. After preincubation for 30 min stimulation was applied by addition of veratrine (76 µM), potassium (56 mM), glutamate (100 and 200 µM) or carbachol (0.1–100 µM) or by application of electrical pulses¹² for 30 min. Pelleted synaptosomes were then exposed to hypo-osmotic lysis at 0 °C in 10 mM phosphate buffer pH 7.0 and the pellet of synaptosomal membranes obtained after centrifugation (100,000g, 10 min) stored at –80 °C. Subsequent receptor binding was performed as described previously¹⁶ using saturating concentrations of [³H] N-methylatropine (10^{–8} M) and a rapid centrifugation method¹⁷. Nonspecific binding, determined by incubating the membranes and ³H-NMA with a high concentration (1 µM) of 3-quinuclidinylbenzilate or atropine, was in general less than 10% of the specific binding. Values for controls were in the range 1–2 nmol ³H-antagonist per g synaptosomal protein. Results are means ± s.e.m. for the number of independent experiments given in brackets.

* Significantly different from control, $P < 0.005$ (using a completely randomised analysis of variance).

80 µg ml^{–1} (equivalent to 20% of the total osmotically releasable synaptosomal protein) to the incubation did not reduce the binding of ³H-NMA to the receptors. A concentration of 150 µg ml^{–1} was required to produce a 15–20% loss in binding over 1 h. The effects shown in Table 1 would require lysis of 50–80% of the incubated synaptosomes on electrical stimulation; such damage has not been observed in previous experiments^{11,12}. As the 'proteolytic potency' of the lysate may have been reduced by extraction and freeze-drying, synaptosomal membranes were incubated in the medium obtained from a previous incubation of intact stimulated synaptosomes. This medium would contain any released soluble enzymes. No loss of binding of ³H-NMA was observed in this experiment. Furthermore, the loss in numbers of muscarinic receptors was not accompanied by a change in activity of the membrane-bound enzymes, Na⁺/K⁺-ATPase or acetylcholinesterase, or any detectable changes in the Coomassie blue staining pattern of the protein bands of solubilised synaptosomal membranes, when electrophoresed on SDS-polyacrylamide gels. In these experiments, electrical stimulation of synaptosomes prelabelled with the irreversible label, ³H-propylbenzylcholine mustard¹⁵, showed no evidence of proteolysis of the specifically labelled 83,000 molecular weight²⁰ binding site of the muscarinic receptor or of release of the membrane-bound receptor into the medium. Thus the observed fall in the antagonist binding capacity seems to be a specific effect linked to the depolarisation event or its sequelae rather than to any gross degenerative effects triggered by the stimulus.

The exposure of crude synaptosome preparations to high concentrations of muscarinic agonists is apparently without effect on the subsequent time-dependent binding kinetics of agonists or antagonists to the receptor population^{16,18}. Our results (Table 1) similarly showed that pretreatment of synaptosomes with carbachol, or glutamate (to simulate transmitter release) or potassium (which is released during depolarisation) had no effect on subsequent ³H-NMA binding.

Our experiments suggest that the decreased binding of ³H-NMA to muscarinic receptors on electrical stimulation or on veratrine treatment is not a direct effect of these agents since the receptor 'loss' is inhibited by low concentrations of tetrodotoxin, which blocks sodium channels. The decreased binding seems to be a result of depolarisation of the synaptosome. One of the main consequences of depolarisation is the release of neurotransmitters. However, the loss of binding activity does not seem to be a result of neurotransmitter–receptor interactions as the phenomenon is not triggered by treatment of the synaptosomes with carbachol or glutamate. Hence the phenomenon is different from the reported agonist-induced loss of β-adrenergic receptors^{6,7}. Furthermore, the fact that K⁺ depolarisation does not decrease ³H-NMA binding suggests that the receptor loss is

specifically associated with the persistent opening of Na⁺ channels.

One possibility is that an agent, released with the neurotransmitter (presumably acetylcholine) modifies the muscarinic receptor. However, any modification of the receptor does not seem to be a general proteolysis; the molecular weight of the receptor and the activity of certain membrane-bound enzymes are not altered by electrical stimulation. The hypothetical factor would have only a transient activity in reducing binding to the receptors in view of the fact that incubation of the medium from electrically stimulated synaptosomes with intact synaptosomes has no effect on ³H-NMA binding. As synaptosomes consist of intact presynaptic structures, commonly carrying parts of the postsynaptic membranes and thickenings, such a factor would be released, along with the neurotransmitter, in high concentration into the synaptic cleft. Any such factor could therefore rapidly modify both postsynaptic and presynaptic receptors before inactivation or diffusion from the cleft.

An alternative possibility that the loss is principally of presynaptic receptors located on the synaptosome membrane and is caused by endocytosis of receptors or a modification of this membrane which is initiated by depolarisation. This hypothesis would imply that at least 50% of the receptors in the preparation were localised presynaptically as this is the observed loss of ³H-NMA binding. There is evidence for the presynaptic localisation of muscarinic receptors^{21,22}: in the present experiments carbachol (200 µM) stimulated amino acid release by 30–80%.

One can speculate that the modification of the binding properties of the muscarinic receptor, if reversible *in vivo*, could provide a mechanism for desensitisation, which is known to occur with considerable facility in muscarinic receptor-linked responses^{23,24}. The mechanism of receptor 'loss' is being investigated further.

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Voltage-dependent calcium current induced by serotonin

CALCIUM ions have a central role in the regulation of neural activity^{1,2}. Changes in intracellular calcium concentration affect membrane permeability to other ions and thereby alter membrane excitability³. Furthermore, calcium is required for transmitter release from nerve terminals⁴. A neurotransmitter that specifically induces an inward calcium current might have profound effects. We report here that in some neurones of *Aplysia*, serotonin can induce such a calcium current. A previous investigation⁵ revealed that iontophoretically applied serotonin elicited an unusual excitatory response in some neurones of *Aplysia*. At hyperpolarised potentials the neurotransmitter produced a slow excitation (component 1) that behaved simply as an increase in sodium conductance, similar to the A' response of Gerschenfeld and Paupardin-Tritsch⁶. At depolarised potentials (>-30 mV), the response was voltage dependent and behaved as a regenerative inward current (component 2). At all potentials, the slow excitatory response to serotonin was insensitive to changes in extracellular chloride or potassium concentration. However, in sucrose-substituted sodium-free solutions, the response was greatly attenuated. Although a calcium current was not excluded, it was tentatively suggested that sodium was responsible for the regenerative inward current as well as for component 1. Further study has now shown that the regenerative inward current component is carried by calcium ions.

The slow excitatory response was elicited by iontophoretic application of serotonin on unidentified neurones of the left caudal quarter of the abdominal ganglion and cells B1 and B2 of the buccal ganglia of *Aplysia californica*. Iontophoretic pulses of serotonin were applied at a constant rate (once every 5 min) throughout the experiment. Neurones were voltage clamped by the single-electrode method of Wilson and Goldner⁷. Methods are the same as those described previously⁵.

Calcium currents in many preparations are blocked by the divalent cations cobalt^{1,8,9} and manganese^{1,9,10}. Figure 1a shows that component 2 of the excitatory response to serotonin is strongly attenuated by 25 mM cobalt in calcium-free seawater. This was consistently observed in all of the seven cells tested with cobalt. Component 1 was affected to varying degrees; in 3 cells it was minimally affected, but in four it was greatly reduced. In the cell illustrated, component 1 was only slightly attenuated. Manganese (25 mM in calcium-free seawater) was also a potent blocker of the excitatory response to serotonin (Fig. 1b). In the six cells tested, it reversibly attenuated both components of the excitatory response to serotonin. The actions of manganese on

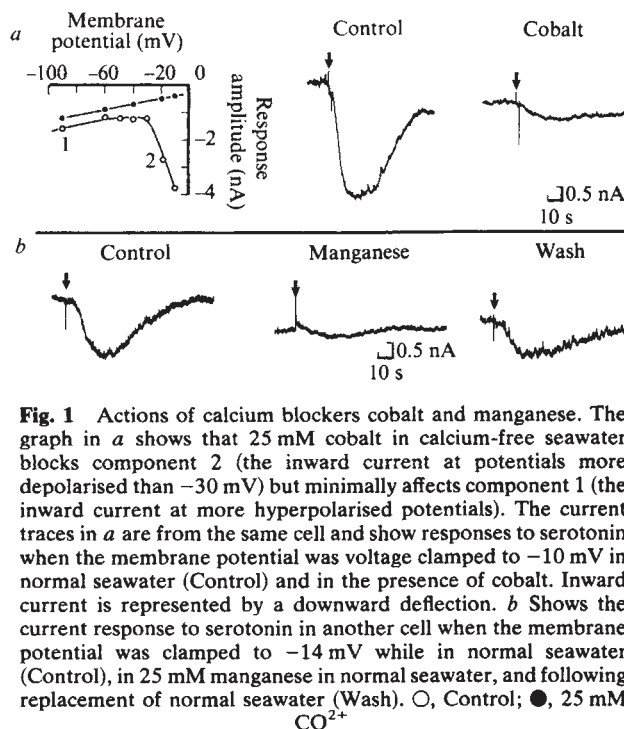


Fig. 1 Actions of calcium blockers cobalt and manganese. The graph in *a* shows that 25 mM cobalt in calcium-free seawater blocks component 2 (the inward current at potentials more depolarised than -30 mV) but minimally affects component 1 (the inward current at more hyperpolarised potentials). The current traces in *a* are from the same cell and show responses to serotonin when the membrane potential was voltage clamped to -10 mV in normal seawater (Control) and in the presence of cobalt. Inward current is represented by a downward deflection. *b* Shows the current response to serotonin in another cell when the membrane potential was clamped to -14 mV while in normal seawater (Control), in 25 mM manganese in normal seawater, and following replacement of normal seawater (Wash). ○, Control; ●, 25 mM CO^{2+} .

component 1 are reminiscent of the effects of this divalent cation on the fast sodium-dependent response to dopamine observed by Ascher¹¹.

When extracellular sodium was replaced with glucosamine, component 1 was attenuated within 15 min. This effect was rapidly reversed with the reintroduction of sodium. As shown in Fig. 2, component 2 was affected differently, in that the response to serotonin was prolonged. In two of the three cells studied, the response was still present after 40-50 min in sodium-free seawater. In the third cell, component 2 of the response was initially prolonged, but within 40 min had declined to zero amplitude. The response recovered slowly in normal seawater, requiring 60 min for 50% reversal. Thus, component 1 is always quickly and reversibly blocked by sodium-free solutions, whereas component 2 is either left unblocked or is very slowly attenuated. In squid axon, prolonged exposure to sodium-free solutions increases intracellular free calcium¹², and intracellular accumulation of calcium has been shown to block an inward calcium current^{13,14}. This may be the basis of our observed depression of component 2 in sodium-free solutions. It was previously reported that the serotonin response was greatly reduced by sucrose-substituted sodium-free seawater⁵. The present results using other Na^+ substitutes suggest that this effect may be related to the decreased ionic strength of the solution or to some nonspecific action, rather than the reduced sodium concentration.

The different ionic sensitivities of the two components of the excitatory response to serotonin indicate that these are independent conductance changes. This is further supported by the observation that in some cells only component 1 was observed, whereas in others only component 2 could be elicited by iontophoresis of serotonin. Component 1 is probably the A' response of Gerschenfeld and Paupardin-Tritsch⁶ and is due to an increase in sodium conductance. The results presented here suggest that calcium is carrying the regenerative inward current at depolarised potentials (component 2).

This is the first demonstration that iontophoretic application of a neurotransmitter can elicit a current carried exclusively by calcium. Of further interest is the regenerative nature of the inward current. Regenerative inward calcium currents have been observed in other systems. A regenerative calcium current is responsible for the calcium component of action potentials¹. It is also thought to underlie the burst firing behaviour of neurones^{15,16}, and Eckert and Lux¹⁵ have suggested that, in

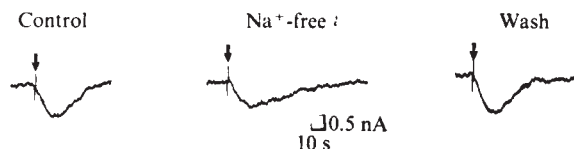


Fig. 2 Effects of sodium-free seawater on current response to serotonin. Na^+ -free trace was taken after 55 min in sodium-free solution. Membrane potential was voltage clamped to -18 mV. Glucosamine HCl completely replaced NaCl in the seawater. The pH of the solution was adjusted to 7.2 by addition of 60 mM $\text{Mg}(\text{OH})_2$. 'Control' and 'Wash' solutions were normal seawater at pH 7.2 to which 60 mM MgCl_2 had been added.

Helix, this current is carried exclusively by calcium. At the presynaptic terminal of squid giant synapse, Katz and Miledi¹⁰ characterised a regenerative inward calcium current that they hypothesised was responsible for transmitter release. Induction of a regenerative calcium current by serotonin may serve to modulate one or more of these processes.

Demonstration of a neurotransmitter-evoked calcium current suggests the possibility of transmitter regulation of intracellular calcium concentration. As intracellular calcium is a principal regulator of a variety of cell functions, this transmitter action is of great physiological significance. Modulation of transmitter release is one possible role for a serotonin-induced calcium current. Our finding may explain some observations reported in the literature. Shimahara and Tauc¹⁷ and Kandel *et al.*¹⁸ have proposed that serotonin mediates presynaptic facilitation. In some cells of *Aplysia*, serotonin can broaden action potentials in the presence of tetraethylammonium, and Klein and Kandel¹⁹ suggested that a calcium current is modulated by the neurotransmitter. Dretchen *et al.*²⁰ have observed that transmitter release at the mammalian neuromuscular junction is enhanced by serotonin, perhaps through a cyclic AMP mechanism. In neuroblastoma–glioma hybrid cells cultured with normal mouse myotubes, Christian *et al.*²¹ have shown that serotonin caused release of acetylcholine and facilitated synaptic release. The regenerative inward calcium current induced by serotonin described here may underlie the presynaptic modulatory actions of serotonin, and may also have other important, as yet unrecognised, regulatory effects on neuronal function.

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Inhibition of Ca^{2+} efflux from mitochondria by nupercaine and tetracaine

It was reported by Mela^{1,2} that local anaesthetics stimulate Ca^{2+} uptake by rat liver mitochondria. This has been attributed to the protective action of these compounds on mitochondrial structure^{3,4}, particularly with regard to inhibition of phospholipase A (refs 5, 6). However, another possibility is that local anaesthetics affect differentially, and more directly, the uptake and efflux of Ca^{2+} . Recent evidence suggests that mammalian mitochondria, in addition to the well documented route for Ca^{2+} accumulation, have a different mechanism for Ca^{2+} release^{7–9}. In the case of rat liver mitochondria, inhibition of Ca^{2+} uptake by the addition of

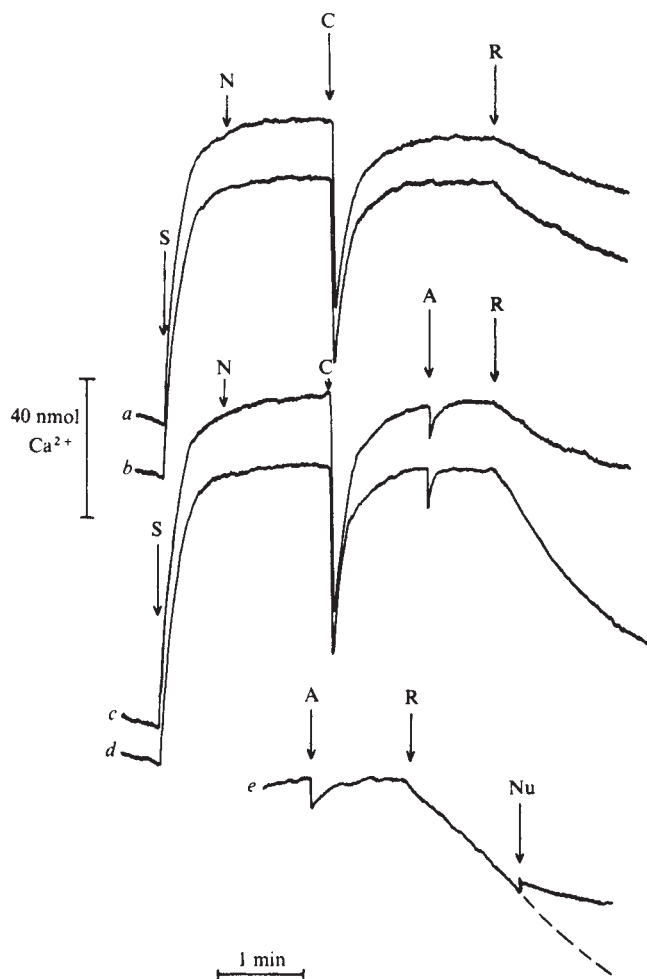


Fig. 1 Effect of nupercaine on rat liver mitochondrial Ca^{2+} uptake and efflux. Each assay contained in a volume of 5 ml: 250 mM sucrose, 20 mM Tris-HCl, pH 7.6, 1 μg rotenone, 8 μM arsenazo III and either 6 mg, (traces a–d) or 4 mg (trace e) of mitochondrial protein. The concentration of free Ca^{2+} indicated by arsenazo III was measured using the absorbance difference 685–665 nm. A downward deflection of the trace indicates increasing extra-mitochondrial Ca^{2+} . The temperature was 30 °C. For traces a–d, succinate (12.5 μmol) was added at S, calcium chloride (50 nmol) at C and ruthenium red (6 nmol) at R. For traces a and c, 0.3 μmol nupercaine was added at N and for traces b and d, 12.5 μmol acetoacetic acid (Li salt) was added at A. For trace e, the experimental technique was exactly as for trace d up to the addition of 12.5 μmol acetoacetate at A. 4 nmol ruthenium red was added at R and 0.5 μmol nupercaine at Nu. The initial part of the trace is omitted for clarity. The dotted line represents the course of Ca^{2+} efflux in the absence of nupercaine.

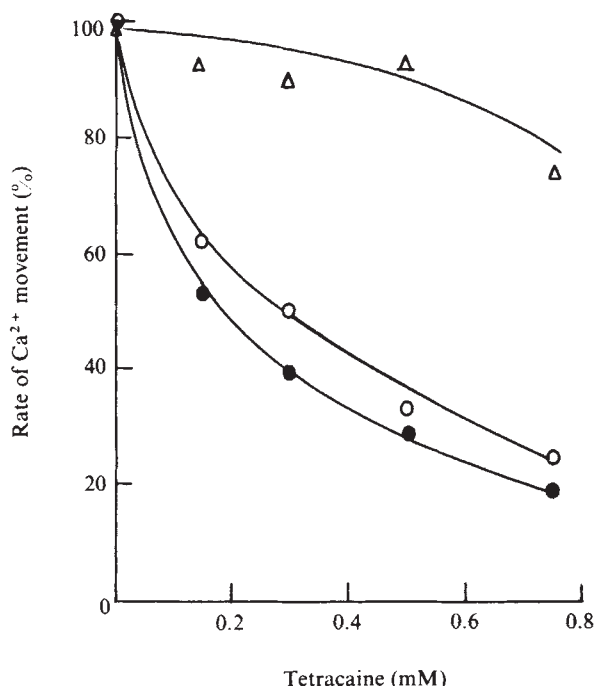


Fig. 2 Effects of tetracaine on Ca^{2+} uptake and efflux. The experimental procedure was the same as for Fig. 1, traces a–d, except that for measurement of the rate of Ca^{2+} uptake, the time axis was expanded $2.5\times$. Each assay contained 8 mg rat liver mitochondrial protein. Δ , Initial rate of Ca^{2+} uptake after the addition of 50 nmol Ca^{2+} ; $\circ$, $\bullet$, initial rate of efflux after addition of ruthenium red. In $\bullet$, 12.5 μmol acetoacetate was added 1 min before ruthenium red.

ruthenium red causes a slow release of Ca^{2+} from the mitochondrial matrix^{7,8} which is enhanced by substrates such as acetoacetate, which cause oxidation of intra-mitochondrial NADH (ref. 10). In mitochondria from other tissues, such as heart and brain^{8,9}, the ruthenium red-insensitive release is dependent on Na^+ ions. We report here, using mitochondria from both rat liver and brain, that nupercaine and tetracaine, at concentrations which do not inhibit Ca^{2+} uptake, cause considerable inhibition of the ruthenium red-insensitive Ca^{2+} efflux observed in energised conditions.

Rat liver mitochondria were prepared as previously described¹¹, except that 0.5 mM EGTA was included in the homogenisation and first-wash media. Non-synaptosomal rat brain mitochondria were isolated by the method of Garbus¹² in a medium containing 320 mM sucrose, 5 mM HEPES-KOH, pH 7.6, and 0.5 mM EDTA, followed by purification on a discontinuous Ficoll gradient as described by Nicholls⁹. Ca^{2+} uptake and output were measured using arsenazo III as an extra-mitochondrial Ca^{2+} indicator¹³ in an Aminco-Chance dual wavelength spectrophotometer equipped with a stirred cuvette. The overall response time was approximately 0.5 s.

Figure 1b shows the standard procedure used for our experiments with rat liver mitochondria. The addition of succinate causes the rapid uptake of free Ca^{2+} from the medium (40 nmol) plus Ca^{2+} which has leaked out of the mitochondria before the initiation of respiration. After 2 min the mitochondria were loaded with a further 50 nmol Ca^{2+} (as CaCl_2). Then, after a further 2 min, uptake was inhibited by the addition of 1 nmol ruthenium red per mg protein. This resulted in a slow Ca^{2+} efflux, as described previously^{7,8}. In Fig. 1a, 60 μM nupercaine was added before Ca^{2+} . It can be seen that this has no effect on the kinetics of Ca^{2+} loading, but the subsequent efflux rate after ruthenium red addition is inhibited to approximately 50% of the control rate. When 2.5 mM acetoacetate is present for 1 min before ruthenium red, the initial rate of efflux is approximately doubled (Fig. 1b, d). Figure 1c shows that this higher rate is also 50% inhibited by 60 μM nupercaine.

Although in these experiments the anaesthetic was in contact with the mitochondria for $3\frac{1}{4}$ min, preincubation is not necessary. Figure 1e shows that the addition of 100 μM nupercaine after efflux has been initiated causes an essentially immediate inhibition of Ca^{2+} output. This result seems to rule out the possibility that effects due to preservation of mitochondrial integrity are involved in the inhibition of efflux, as these would presumably require the presence of the anaesthetic throughout the experiment.

Figure 2 summarises data for tetracaine, including its effect on the velocity of Ca^{2+} uptake. It can be seen that concentrations of the anaesthetic which cause up to 70% inhibition of efflux have essentially no effect on uptake, although at higher concentrations uptake is impaired to some extent. Preliminary observations with butacaine suggest that it behaves similarly to tetracaine, but there is a less clear distinction between its effects on uptake and efflux. The concentration of butacaine required for 50% inhibition of efflux is ~ 0.8 mM, compared with 0.2 mM for tetracaine and 0.06 mM for nupercaine.

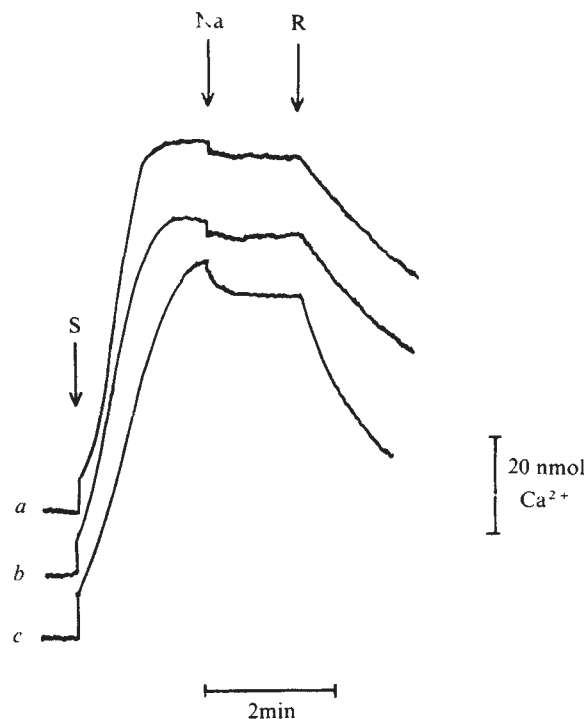


Fig. 3 Effects of nupercaine and tetracaine on rat brain mitochondrial Ca^{2+} uptake and efflux. Each assay contained in 5 ml: 250 mM sucrose, 20 mM Tris-HCl, pH 7.6, 1 μg rotenone, 1 mM potassium phosphate, pH 7.6, 6 μM arsenazo III, 12 μM free Ca^{2+} (measured with a Ca^{2+} -sensitive electrode) and 2.4 mg mitochondrial protein. For all traces: S indicates the addition of 12.5 μmol succinate (K^+ salt); Na, 25 μmol NaCl; R, 3 nmol ruthenium red. In addition, experiment a contained 1.1 μmol nupercaine and b 2.5 μmol tetracaine, both added 30 s before succinate. Other conditions were as for Fig. 1.

Figure 3 shows the effects of nupercaine and tetracaine on Ca^{2+} transport by rat brain mitochondria. In the conditions of this experiment, both compounds produce a considerable stimulation of uptake, an almost complete inhibition of the Na^+ -induced efflux and a 50% inhibition of the rate of Ca^{2+} release subsequent to ruthenium red addition. The nature of the stimulation of uptake is not clear, but one possibility is a decrease in Ca^{2+} cycling during uptake.

Because the local anaesthetics used in this study all carry a positive charge, it might be expected that when they bind to a membrane they would reduce its passive permeability to cations¹⁴. Also, local anaesthetics have been shown to compete with Ca^{2+} for binding sites in the mitochondrial membrane¹⁵. It is rather surprising, therefore, that, at low concentrations,

nupercaine and tetracaine inhibit Ca^{2+} efflux and have little effect on, or stimulate, Ca^{2+} uptake. Also, we have found that the rapid efflux of Ca^{2+} brought about by collapse of the membrane potential by uncouplers or antimycin A (ref. 7) is not affected by tetracaine, either in the presence or absence of ruthenium red. The inhibition of efflux in energised conditions thus seems to be a relatively specific effect, and provides further evidence for the view that the route for Ca^{2+} efflux from mitochondria is different from that for uptake. It is clearly a possibility that if mitochondria are involved in the regulation of cytosolic Ca^{2+} concentration, then this concentration might be perturbed by the application of nupercaine or tetracaine. Such a perturbation of external Ca^{2+} concentration would not be readily observed in the experimental conditions shown in Fig. 1, as even in the absence of nupercaine, measurements with a Ca^{2+} -sensitive electrode show that the mitochondria reduce the free external Ca^{2+} concentration to approximately 1 μM . If nupercaine stimulated the uptake of some, or even all, of this residual Ca^{2+} , the resulting change in absorbance of arsenazo III would be very small. It has, however, been observed¹⁶ that the application of 0.1–1 mM tetracaine to perfused liver blocks both glucagon and cyclic AMP stimulated gluconeogenesis and Ca^{2+} release into the perfusate.

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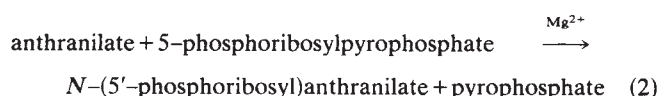
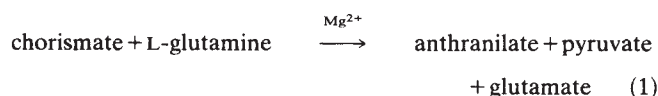
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Gene fusion during the evolution of the tryptophan operon in Enterobacteriaceae

THE genes of tryptophan biosynthesis are organised differently in various species of microorganisms¹. In several organisms it seems that gene fusions have joined functionally distinct polypeptide chains into single bifunctional proteins. Here we present our analysis of one example of a possible gene fusion and an attempt to deduce the sequence of events responsible.

The two initial reactions specific to the tryptophan pathway



are catalysed by the enzymes anthranilate synthetase (AS) and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyl transferase (PRT) respectively. In many bacterial genera^{1,2}, AS is an enzyme complex containing two non-identical polypeptides, components I and II³. Component I alone can convert chorismate to anthranilate using NH_3 but not L-glutamine as the amino donor. Component II contributes glutamine amidotransferase (GAT) activity to the complex, thus conferring the ability to use L-glutamine as the amino donor². While the size of AS component I is fairly uniform in bacteria², at least two different forms of component II have been observed. In *Escherichia coli*⁴, and *Salmonella typhimurium*⁵ it has a molecular weight of about 65,000 and has two distinct enzymatic capabilities: it provides the GAT activity for reaction (1) when complexed with component I, and catalyses reaction (2), the PRT reaction, whether in the complex or not. In *Serratia marcescens* a small polypeptide with a molecular weight of 21,000 contributes the GAT activity to AS^{6–8}, but a separate polypeptide with a molecular weight of 45,000^{8,9} has PRT activity.

The two enzyme activities of AS component II of *E. coli* and *S. typhimurium* are associated with different segments of the polypeptide chain. Genetic studies indicate that the GAT activity resides in the N-terminal third of the molecule while PRT activity is associated with the remainder of the polypeptide chain^{10–12}. Mild proteolytic digestion of intact component I–component II complexes of *E. coli* or *S. typhimurium*^{8,13} produces a component II fragment of molecular weight 23,000 which retains GAT activity but lacks PRT activity. Li *et al.*⁸ have found extensive amino acid sequence homology at the N-terminal end of these component II fragments of *E. coli* and *S. typhimurium* and the functionally homologous intact component II GAT polypeptide of *S. marcescens*. This suggests that the GAT region of the bifunctional component II polypeptides of *E. coli* and *S. typhimurium* and the smaller GAT subunit of *S. marcescens* share the same evolutionary origin and lends direct support to the idea that the bifunctional component II polypeptides of *E. coli* and *S. typhimurium* arose by fusion of two separate genes, one coding for a GAT and a second coding for PRT^{5,8,11,14}.

In *E. coli* all the genes of tryptophan biosynthesis are organised as an operon with the structural gene for AS component I (*trpE*) closest to the promoter/operator region¹⁵. *trpE* is followed by the structural gene for AS component II (*trpD*), with the genetic region specifying the GAT portion of the polypeptide (*trpG*) closest to *trpE*⁸. In order to indicate the two domains of the structural gene for the bifunctional component II polypeptide of *E. coli*, we shall designate it *trp(G)D* (ref. 1). In *S. marcescens*, the evidence for the organisation of the genes of tryptophan biosynthesis as an operon is indirect⁹. In particular, it was uncertain whether the structural gene for the GAT subunit of AS, *trpG*, lies adjacent to *trpD*, the PRT structural gene, or whether it is unlinked to the remaining genes of the operon, as in *Bacillus subtilis*, where AS component II also seems to provide GAT function for *p*-aminobenzoate synthesis^{16,17}.

We have recently characterised a cloned DNA fragment containing the regulatory region and first structural gene, *trpE*, of the *S. marcescens* *trp* operon¹⁸. DNA sequence analysis revealed that the fragment also contains the sequence coding for the first nine amino acids at the N-terminus of AS component II, confirming that *trpG* lies adjacent to *trpE* in the *trp* operon of *S. marcescens*¹⁸. Here we report the cloning of a different DNA fragment containing all the structural genes of the *S. marcescens* *trp* operon with the exception of *trpA*. A functional and structural analysis of this DNA fragment established that the gene order at the promoter proximal end of the *S. marcescens* *trp* operon (*trpE*–*trpG*–*trpD*) is analogous to the arrangement in *E. coli*. We have determined the DNA nucleotide sequence of the *trpG*–*trpD* punctuation region in *S. marcescens* and of the analogous region of the fused *trp(G)D* gene of *E. coli*. A comparison of the two sequences reveals extensive homology

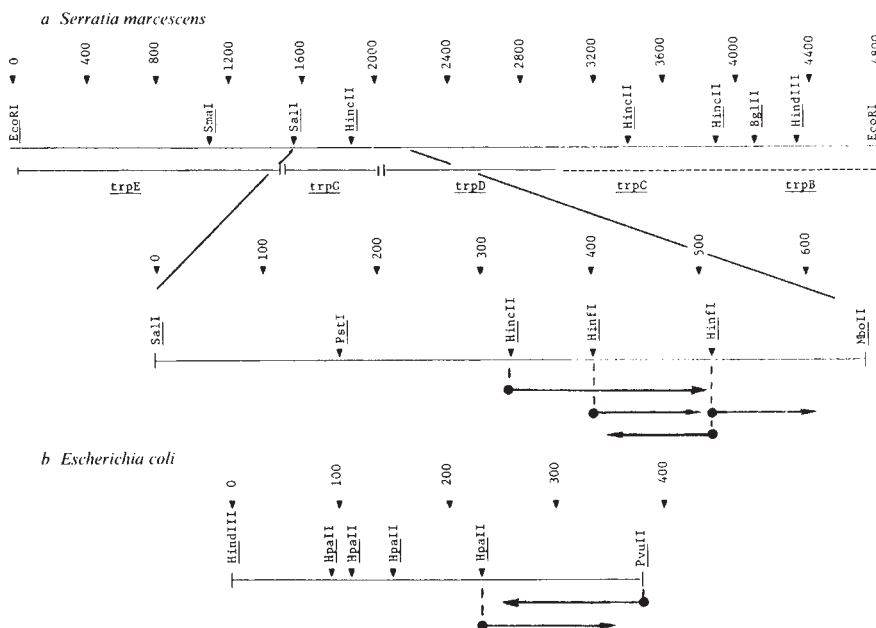


Fig. 1 *a*, Restriction map of a cloned fragment containing *S. marcescens* *trpEDCB*. Chromosomal DNA of *S. marcescens* was prepared by the method of Saito and Miura³⁷. 10 µg of chromosomal DNA and 5 µg of pBR322 DNA were digested with *EcoRI*, phenol extracted, ethanol precipitated and resuspended in 250 µl of ligation buffer (60 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 10 mM dithiothreitol, 1 mM ATP). The mixture was incubated in the presence of T4 DNA ligase for 2 h at 13 °C. Ligation was stopped by ethanol precipitation and the pellet resuspended in 100 µl TE-buffer (10 mM Tris-HCl, pH 7.9, 0.1 mM EDTA). 20 µl were used to transform *E. coli* strain JA 221(C600 *r⁻m⁺ΔtrpE5 leu recA*, obtained from J. Carbon) to prototrophy. Trp⁺ transformants were screened for plasmid content. Plasmid pGM6 was recovered from such a transformant. It carries a single 4,800 base pair *EcoRI* insert. A restriction map of this insert is shown in the top portion of (*a*). The direction of the transcription is from left to right. The approximate location of the various *trp* genes is indicated underneath; the gene order in the dotted portion of the line has not been determined with certainty. The bottom portion of (*a*) shows an expanded restriction map of the DNA region containing the *trpG*-*trpD* interconnecting/intercistronic region. The arrows indicate the direction and length of sequencing runs, *b*, Restriction map of a DNA fragment from *E. coli* containing the *trpG*-*trpD* interconnecting region. *E. coli* *trpPOLED* DNA was isolated from plasmid pVH153²⁰. The arrows underneath the restriction map indicate the length and direction of sequencing runs.

within the *trpG* and *trpD* structural genes, but considerable divergence in the *trpG*-*trpD* junction region. How the two sequences may be evolutionarily related will be considered.

Chromosomal DNA of *Serratia marcescens*, obtained from wild-type strain SM6, was restricted with endonuclease *EcoRI* and the cleavage products were inserted into the *EcoRI* site of plasmid pBR322¹⁹. Plasmids carrying the initial portion of the *S. marcescens* *trp* operon were selected from the ligation mixture by their ability to transform an *E. coli* recipient strain which carries a deletion of most of *trpE* to tryptophan independence (see legend to Fig. 1 for experimental details). Plasmids pGM6, isolated from such a transformant, carries a single *EcoRI* insert of approximately 5,000 base pairs, whose restriction map appears in the top portion of Fig. 1*a*. pGM6 yields Trp⁺ transformants with *trpE*, *trpD*, *trpC* and *trpB* but not *trpA* mutant recipients. Thus pGM6 carries most of the *S. marcescens* *trp* operon. The physical location and orientation of the *S. marcescens* *trp* genes on the *EcoRI* insert was determined by subcloning portions of the insert using various restriction enzymes. One such plasmid, pGM63, carries the 1,500-base pair *EcoRI*-*SalI* segment from the left hand end of the *EcoRI* fragment shown in Fig. 1 (ref. 18). This DNA fragment contains the entire *trpE* gene from *S. marcescens*¹⁸. The *EcoRI* site precedes the start of *trpE* by 20 base pairs, while the *SalI* site is located approximately 30 base pairs beyond the *trpE*-*trpG* boundary¹⁸. Neither pGM6 nor pGM63 contains the promoter/operator region of the *S. marcescens* *trp* operon. Therefore, in both plasmids transcription of the *trp* genes must originate at a pBR322 promoter. The orientation of the insert is the same in both plasmids. Transcription is towards the region determining tetracycline resistance and away from the region determining ampicillin resistance¹⁹.

We estimated the location of the *trpG*-*trpD* boundary of *S. marcescens* on the basis of the molecular weight of 21,000 for

the *trpG* gene product^{6,8}. The end of *trpG* and the presumed *trpG*-*trpD* intercistronic region was predicted to be approximately 500 base pairs from the *SalI* site in the initial portion of *trpG*. A detailed restriction map of this DNA segment is shown in the bottom portion of Fig. 1*a*. DNA sequence analysis was performed with DNA fragments labelled at either the *HinfI* sites (positions 400 and 520) or the *HincII* site (position 330) as outlined in Fig. 1*a*.

The analogous region of the *E. coli* *trp* operon is contained on plasmid pVH153²⁰. This plasmid carries *E. coli* *trpPOLE(G)D* on a 4.7 megadalton *EcoRI* fragment from λ *trpED*₁₀ inserted into the *EcoRI* site in pVH51 (mini-ColEI²¹). A *HindIII* site is located in the initial portion of *E. coli* *trp(G)D*²². The exact location of this restriction site, 275 base pairs from the *trpE*-*trp(G)D* punctuation region, has been determined by DNA sequence analysis (C. Yanofsky *et al.*, in preparation). The molecular weight of 23,000 for the amino terminal proteolytic fragment of *E. coli* AS component II⁸ is very similar to that of component II from *S. marcescens* (21,000). By analogy to the *S. marcescens* *trp* operon we would therefore expect the *trpG*-*D* junction region in *E. coli* to be located approximately 300 base pairs downstream from the *HindIII* site. A restriction map of the corresponding DNA segment is shown in Fig. 1*b*. DNA sequence analysis was performed with DNA fragments labelled at either the *HpaII* site at position 225 or the *PvuII* site at position 380 as indicated in Fig. 1*b*. The nucleotide sequence of this DNA segment is presented in Fig. 2 along with the sequence of the analogous region from *S. marcescens*.

The *S. marcescens* nucleotide sequence contains a potential ATG translation initiation codon at positions 88-90 (Fig. 2). The predicted amino acid sequence from this site is identical to the previously determined amino acid sequence at the N-terminal end of *S. marcescens* PRT²³. This confirms our assumption that *trpD* follows *trpG* in the *S. marcescens* *trp*

operon. The amino acid sequence at the C-terminal end of *S. marcescens* AS component II was determined by carboxypeptidase digestion of purified component II. The results [(Trp, Ala)-Leu-Ala-Lys-COOH] indicate that the TAA at positions 70-72 is used as the translation stop codon. In *S. marcescens* *trpG* and *trpD* are thus separated by an untranslated region 16 base pairs in length. Six contiguous nucleotides within this region (TAAGGA) are homologous to the 3' terminus of *E. coli* 16S rRNA²⁴, a feature common to many prokaryotic ribosome binding sites²⁵.

The nucleotide and amino acid sequences preceding and following the segment corresponding to the *trpG-trpD* intercistronic region have been extensively conserved between *S. marcescens* and *E. coli*. Twenty of 23 amino acids in the *trpG* portion and 16 of 22 amino acids in the *trpD* portion are identical (Fig. 2). Several changes at the nucleotide level (eight in *trpG*; seven in *trpD*) are not reflected at the amino acid level. In contrast to this, sequence conservation is not immediately apparent in the *trpG-trpD* intercistronic region (Fig. 2). In *E. coli* 15 nucleotides are present in this DNA segment compared with 16 in *S. marcescens*; as a consequence the coding sequences of the *E. coli* *trpG* and *trpD* segments are in phase. This, together with the absence of translational stop and start signals (also there is no obvious sequence homology to the 3' end of 16S ribosomal RNA), accounts for the production of a 'fused' *trp(G)D* polypeptide in *E. coli*.

The marked similarity between the two sequences suggests a common origin. This implies that at least once during the

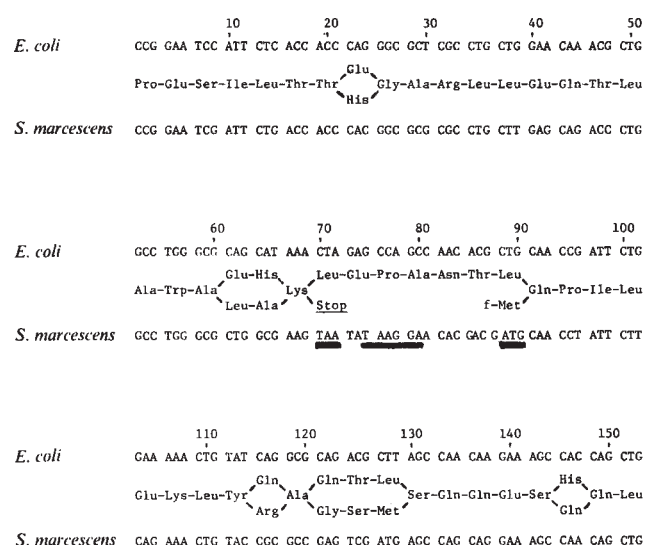


Fig. 2 Nucleotide sequence of the DNA region surrounding the *trpG-trpD* interconnecting/intercistronic region in *E. coli* and *S. marcescens*. The two sequences have been lined up in the *trpG* and *trpD* portion to show homologies. The predicted amino acid sequence is indicated between the DNA sequences. A split amino acid sequence indicates that the nucleotide sequence predicts a different amino acid for the two organisms at that position. The predicted amino acid sequence following the ATG codon at position 88-90 of the *S. marcescens* nucleotide sequence corresponds exactly to the previously determined amino acid sequence at the N-terminal end of *S. marcescens* PRT²³. The C-terminal residues of *S. marcescens* AS component II were determined by carboxypeptidase A and B digestions of the purified protein^{8,35}. The sequential appearance of Lys, Ala, Leu, and Trp served to identify the TAA at position 70-72 as the translation stop codon. The message punctuation signals in the *S. marcescens* *trpG-D* intercistronic region (translation stop and start codons, homologies to the 3' end of *E. coli* 16S rRNA) are underlined. DNA sequence analysis was performed by the method of Maxam and Gilbert³⁶. Details of this and related procedures such as isolation of plasmid DNA and DNA restriction fragments, restriction site mapping, phosphatase treatment, polynucleotide kinase reactions and agarose and polyacrylamide gel electrophoresis are given in refs 38, 39.

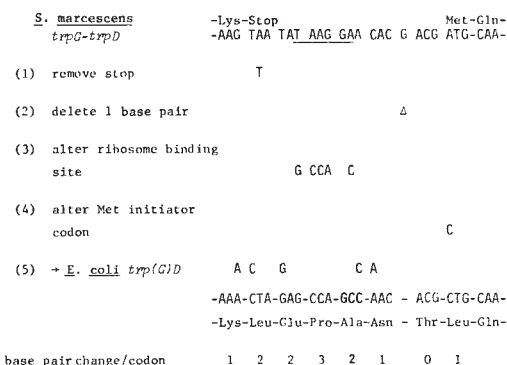


Fig. 3 Hypothetical sequence of events involved in the conversion of the separate *trpG* and *trpD* genes of *S. marcescens* into the single fused *trp(G)D* gene of *E. coli*. In reality the postulated fusion event would have taken place in a common ancestor of both organisms which may have been similar to *S. marcescens* in its nucleotide sequence.

evolution of the *trp* genes in the Enterobacteriaceae a gene fusion or gene separation event occurred in the *trpG-trpD* region. Bonner *et al.*²⁶ have suggested that evolutionary development progresses from independent proteins through multimeric enzymes to fused gene products. Alternatively, as pointed out by Largen *et al.*²⁷, a fusion event involving ancestral *trpG* and *trpD* genes may have occurred before the appearance of the Enterobacteriaceae, in which case the separate *trpG* and *trpD* genes of *S. marcescens* would represent the result of a subsequent separation of the fused genes. Although the first possibility seems more plausible, the available evidence does not allow us to rule out the alternative route.

If the fused *trp(G)D* arrangement of *E. coli* were ancestral to the *S. marcescens* gene order, the conversion of the first Lys codon of Fig. 3 to a stop codon would probably suffice to give separate, active, *trpG* and *trpD* polypeptides. The present day *trpG-trpD* interconnecting nucleotide sequence of *E. coli* may in fact have weak ribosome binding and translation initiation activity. Mutational studies suggest that there is a translation reinitiation site within *E. coli* *trp(G)D* which can produce a polypeptide with PRT activity^{10,12}. In addition, as mentioned earlier, the polypeptide segment specified by the *trpG* portion of *trp(G)D* of *E. coli* has GAT activity. Thus separation could occur without loss of activity. Subsequent mutational changes could improve the efficiency of ribosome binding to the initiation region of *trpD* and consequently increase the yield of PRT.

One of several hypothetical sequences of events that would result in the formation of the fused *E. coli* *trp(G)D* gene from the separate *trpG* and *trpD* genes of *S. marcescens* is illustrated in Fig. 3. In a first step a single mutational event removes the *trpG* translational stop signal, allowing out-of-phase translation into the adjacent *trpD*. Both *trpG* and *trpD* are still expressed but out-of-phase translation probably would reduce production of the *trpD* polypeptide. Deletion of a single base pair in the immediately adjacent region (step 2) results in the formation of a fused polypeptide whose two functional domains would be held together by an 'immature' connector region. However, the fused gene retains the efficient parental *trpD* ribosome binding site and thus might produce both a fused *trp(G)D* polypeptide and a *trpD* polypeptide. Subsequent mutations in the intercistronic region could inactivate the ribosome binding site and/or introduce functionally acceptable amino acids (step 3) and alter the ATG start sequence (step 4). Three synonymous codon changes and two substitutions resulting in amino acid replacements (step 5) would then give the *E. coli* sequence. The *E. coli* connector region presumably does not interfere with the correct folding of the two functional domains of the fused polypeptide. As can be seen from the last line of Fig. 3, the overall sequence of events would not involve extensive base changes in the intercistronic region. This sequence of events neglects the fact that the fusion event would have taken place in an unknown *Serratia*-like

ancestor which differed from *S. marcescens* in its nucleotide sequence. It is obvious that a single deletion event could more simply fuse the adjacent *G* and *D* genes. Perhaps the connector region of *E. coli*, in mRNA or protein, has some important unsuspected function.

Gene fusion and separation may have occurred several times in the evolution of the tryptophan genes. In yeast the third and fourth steps of the biosynthetic pathway are catalysed by separate proteins coded for by unlinked genes²⁸, while in *E. coli* and many other prokaryotes both reactions are catalysed by a single bifunctional polypeptide encoded in a single gene, *trpC*^{1,28,29,30}. The converse is found for tryptophan synthetase, where the fungal enzyme consists of a fusion of α and β chain elements^{31,32} which are separate polypeptides in *E. coli* and other prokaryotes³³. The *trpG-trpD* case may be unique in that both the fused and separate states of the genes are found within closely related organisms, where evolutionary divergence apparently has not been significant enough to totally obscure the original fusion event.

If the fusion of genes involved in the same pathway confers a selective advantage, one wonders why in *S. marcescens* the separate *trpG* and *trpD* genes have not been replaced by a fused genetic element, particularly since fusion need involve so few mutational events. It is conceivable that in order for the fusion to become fixed, compensatory mutations must occur elsewhere within *trpG* or *trpD*. In such circumstances, the probability of a successful fusion event would be substantially reduced. Alternatively, *S. marcescens* AS component II may serve as a GAT subunit in *p*-aminobenzoate formation as does AS component II of *B. subtilis*^{16,17} and *Acinetobacter calcoaceticus*³⁴. Such a dual function of the *trpG* gene product would obviously place severe restrictions on the likelihood that *trpG* would undergo gene fusion.

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Specific binding of Mu repressor to DNA

PHAGE MU is a temperate virus of *Escherichia coli* (for reviews see refs 1, 2). The immunity system of Mu has been shown by genetic and physical mapping to lie in the furthest left, 1,000-base-pair restriction fragment of the phage DNA³⁻⁶. This end of the phage genome is associated with a short (100-base pair) segment of bacterial DNA⁴. The *HindIII*-c restriction fragment of Mu (the furthest left 1,000 base pairs⁴) has been cloned into the amplifiable plasmid pMB9 (ref. 7). These recombinant plasmids confer high levels of Mu immunity on their hosts and, when recombined into minicell-producing strains, were shown to produce an additional protein of 25,000 subunit molecular weight in purified minicells^{6,7}. This protein is assumed to be the Mu repressor because its production is correlated with immunity to Mu. We report here experiments which show that this protein binds specifically and with high affinity to Mu DNA, as would be expected of the Mu repressor. Furthermore, we demonstrate that the DNA sequence (operator) to which the Mu repressor binds is located on the same *HindIII*-c restriction fragment of Mu DNA as the repressor gene.

In these studies DNA binding was assayed using the unpurified crude extract of labelled minicells as a source of repressor. These extracts contain so few other labelled proteins that the only purification required is the removal of the plasmid DNA. Extracts of minicells derived from strains transformed

Table 1 Specific binding of the temperature-sensitive Mu repressor to Mu DNA

Crude lysate of repressor	DNA-bound repressor (fractions 5/6 (6/7*))	Unaggregated repressor (fraction 9/10)
-DNA	<1%	31%
+ Mu DNA	13%	26%
+ D108 DNA	<1%	31%
+ λ DNA	<1%	31%
+ 25 DNA	14%*	23%
+ p5D7 DNA	<1%	7%

The crude lysate of minicells was prepared as described in the legend to Fig. 1, and the top fraction of the high salt gradient used as the source of repressor. Aliquots were mixed with buffer IV or buffer IV plus 20 μ g Mu, λ or D108 DNA, or 2.0 μ g linearised plasmid 25 DNA or plasmid p5D7 DNA. The DNA concentrations were such that approximately equal numbers of DNA molecules were added to each binding mix. The binding mixture was incubated and run on gels as described in Fig. 1 legend. The fractions collected were assayed for proteins and DNA as before. Autoradiographs of the SDS-acrylamide gels run on gradients with repressor alone, repressor + Mu, repressor + D108 and repressor + λ DNA are shown in Fig. 1. The autoradiographs were scanned and the peaks of repressor weighed and normalised as described in Fig. 2 legend. The per cent of total repressor recovered from fractions 5+6, or 6+7 (DNA-bound repressor), and from fractions 9+10 (unaggregated repressor) is shown. Due to the noise in the system, repressor in amounts <1% of the total repressor in the gradient would not be detectable by scanning; Mu, D108, λ and plasmid p5D7 DNA sediment to fractions 5 and 6 in the gradients, whereas plasmid 25 DNA linearised by digestion with *HindIII* sediments to fractions 6 and 7 in these conditions.

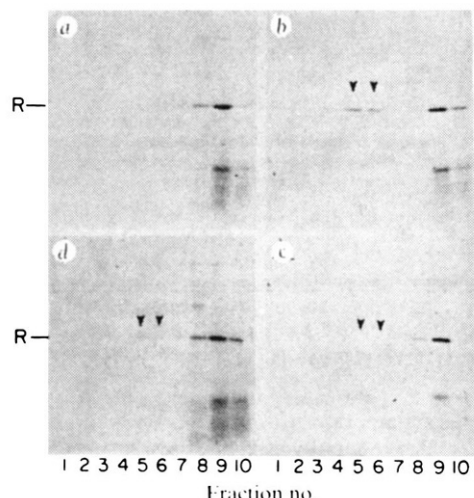


Fig. 1 Comparison of repressor binding to Mu, D108 and λ DNA. ^{35}S -Methionine-labelled minicells purified (according to the procedure described by Zipser *et al.*⁶) from $\chi 1274/\text{pMB9-mu } c1ts62 \text{ HindIII-c } 25$ (frozen in 10 mM Tris, pH 8, 5 mM EDTA, 10% sucrose, 0.05 M NaCl) were lysed in the following manner. The minicells were thawed slowly on ice, and NaCl and lysozyme were added to final concentrations of 0.3 M NaCl and 150 $\mu\text{g ml}^{-1}$ lysozyme. After 1 h on ice, the minicells were frozen in a dry ice-ethanol bath and thawed on ice, five times. Triton X-100 was added to a final concentration of 0.33% (v/v), and the minicells were incubated for 20 min at 0°C. The crude lysate (150 μl total volume) was centrifuged at 20,000 r.p.m. in a Beckman SW 60 rotor to remove cell debris and insoluble proteins. The supernatant was adjusted to 0.6 M NaCl and layered onto a 5 ml 5–20% sucrose in buffer IV (10 mM Tris, pH 8, 1 mM EDTA, 0.1 mM dithiothreitol, 50 $\mu\text{g ml}^{-1}$ bovine serum albumin) gradient containing 0.6 M NaCl. The gradient was then centrifuged for 2 h at 45,000 r.p.m. (4°C) in the Beckman SW 50.1 rotor, and 10 0.5-ml fractions were collected from the bottom of the gradient. Sedimentation through high salt was done to eliminate variability in the binding assay due to repressor binding to endogenous DNA. In these conditions, the repressor and other labelled proteins remain at the top of the gradient, whereas endogenous DNA moves to the middle of the gradient. The top fraction was used as the source of repressor in the binding assay. Samples (0.1 ml) of the top fraction were mixed with buffer IV or buffer IV plus 20 μg DNA (Mu, D108 or λ), and adjusted to 0.2 M NaCl. The binding mixtures were incubated for 30 min at 0°C and then layered onto 5 ml 5–20% sucrose in buffer IV gradients containing 0.05 M NaCl. The gradients were centrifuged for 2 h at 45,000 r.p.m. (4°C) in a Beckman SW 50.1 rotor, and 10 0.5-ml fractions were collected from the bottom of each gradient. Samples from each fraction were subjected to electrophoresis on 17.5% SDS-polyacrylamide gels. The gels were autoradiographed to assay for labelled proteins. Samples from the gradient fractions were also subjected to electrophoresis on 1% agarose gels. These gels were stained with ethidium bromide and photographed under UV light to assay for DNA. Autoradiographs of the SDS-polyacrylamide gels run on gradients with a, repressor alone; b, repressor plus Mu DNA; c, repressor plus D108 DNA; and d, repressor plus λ DNA are shown above. The arrow labelled R indicates the position of the repressor band on the gels. The vertical arrows indicate the position of the DNA in the gradients. Mu and λ DNA were prepared from lysogens carrying Mu $c1ts62$ and λ $c1857$ S7, respectively, by the procedure of Kahmann¹². D108 was prepared in a similar manner, except that purification of phage was done after infection of strain 783 (Thi⁻, Trp⁻, su⁺) with wild-type D108.

with plasmids carrying the Mu repressor gene were mixed with unlabelled DNA and then sedimented through sucrose gradients^{8,9}. To assay repressor, samples from each fraction of the gradients were subjected to electrophoresis on SDS-polyacrylamide gels, and the amount of repressor determined from autoradiographs of these gels. The position of the DNA in the gradient is determined from ethidium bromide-stained agarose gels run on each gradient fraction.

The specificity of Mu repressor is demonstrated in Fig. 1 and Table 1. When a repressor extract without DNA is sedimented through a gradient, the repressor, as well as other labelled proteins, remain near the top of the gradient. When the same extract is used with Mu DNA, a significant fraction of the repressor sediments with the DNA, but the position of other labelled proteins remains unchanged. If equal amounts of DNA from D108, a phage heteroimmune to Mu, or from phage λ are used, only trace amounts of Mu repressor are bound.

When the binding was carried out at 0.1 M rather than 0.2 M NaCl, some repressor was associated with D108, but less than the amount associated with Mu DNA. More repressor was found in the fractions between the DNA peak and the top of the gradient with D108 DNA than was found with Mu DNA, suggesting that the repressor was dissociating from D108 DNA as the DNA sedimented through the gradient. This indicates that although repressor binds tightly to Mu DNA, it has some affinity for other DNAs at lower salt concentrations.

Increasing the salt concentration in the binding reaction from 0.1 to 0.3 M NaCl had little effect on the amount of repressor bound to Mu DNA, but increasing it to 0.6 M NaCl eliminated repressor binding to Mu DNA. Binding was also not detected when both the binding mixture and the gradient contained 0.2 M NaCl.

Phage D108 is closely related, but heteroimmune, to phage Mu¹⁰. Heteroduplex analysis of D108 and Mu shows a total nonhomology of 6% between the two phages, the largest region of nonhomology (3%) being in the left end of the phages¹⁰, where the repressor maps. As D108 does not bind Mu repressor, the operator must be nonhomologous and could be in the left end of the phage genome also. This would be similar to the situation found with bacteriophage λ , where the operators to which the λ repressor binds are located close to the repressor gene^{8,11}. This might mean that the Mu operator is also on the cloned *HindIII-c* fragment that carries the repressor gene.

To test this possibility DNA of plasmid 25 (the same plasmid used to synthesise repressor) was used in the binding assay. As shown in Fig. 2, in the same conditions which gave specific binding to Mu DNA, the repressor binds to linearised plasmid 25, but not to linearised pMB9 DNA. Furthermore, the repressor does not bind to DNA of plasmid p5D7 (a recombinant plasmid constructed by the terminal transferase tailing method in a fashion analogous to that of plasmid 25 but inserting yeast genes instead of Mu DNA). This argues against the possibility that the repressor binds to the AT-rich regions created during cloning (see Table 1). Three other plasmids (36, 42 and 54 (ref. 7)) which were constructed in the same manner as plasmid 25 and which carry the same *HindIII* fragment of Mu $c1ts62$, also show specific binding of the Mu repressor. Thus, an operator to which the Mu repressor binds, is located in the furthest left 1,000 base pairs of the Mu genome.

The crude repressor preparations used for these studies contain varying amounts of inactive repressor (repressor which cannot bind to operator-containing DNA). The amount of

Table 2 Binding of the temperature-sensitive Mu repressor to varying amounts of DNA

Crude lysate of repressor	DNA-bound repressor (fractions 6/7)	Unaggregated repressor (fractions 9/10)
– DNA	<1%	48%
+ 0.1 μg 25 DNA	28%	44%
+ 0.5 μg 25 DNA	55%	47%
+ 2.5 μg 25 DNA	81%	38%
+ 12.5 μg 25 DNA	62%	35%
+ 0.5 μg pMB9 DNA	9%	49%

A crude lysate of minicells was prepared as described in Fig. 1 legend. Aliquots of the top fraction of the high salt gradient were mixed with buffer IV, or buffer IV plus varying amounts of linearised plasmid 25 DNA or 0.5 μg linearised plasmid pMB9 DNA, and adjusted to 0.2 M NaCl. Binding mixtures were incubated and run on gradients as before. The fractions collected were assayed for proteins and DNA as described in Fig. 1 legend. Autoradiographs of the acrylamide gels were scanned for the repressor band and the peaks weighed and normalised as described in Fig. 2 legend. The per cent of total repressor recovered in fractions 6+7 (DNA-bound repressor) and in fractions 9+10 (unaggregated repressor) is shown above. Due to the noise in the system, repressor in amounts <1% of the total would not be detectable by scanning. The DNA sediments to fractions 6 and 7 in these conditions.

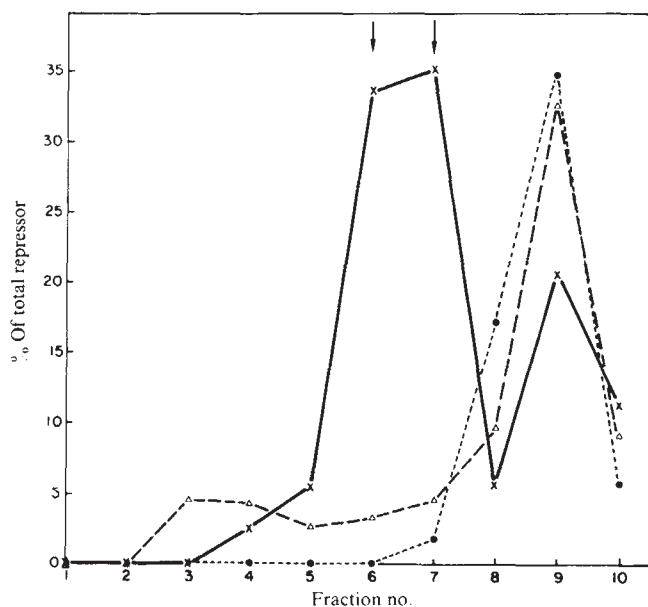


Fig. 2 Comparison of repressor binding to plasmid 25 and pMB9 DNA. A crude lysate of minicells was prepared as described in the legend to Fig. 1. As before, the top fraction of the high salt gradient was used as the source of repressor. Aliquots were mixed with buffer IV or buffer IV plus 2.5 μ g of linearised plasmid 25 or pMB9 DNA, and adjusted to 0.2 M NaCl. The binding mixture was incubated and run on gradients as described in Fig. 1 legend. The fractions collected were assayed for proteins and DNA as before. The repressor band in autoradiographs of the acrylamide gels were scanned using a Joyce, Loebel densitometer (Chromoscan Mk. II). The peaks were cut out and weighed to determine the amount of repressor in each fraction. To normalise the amount of repressor in each fraction, samples of the binding mixtures before sedimentation were subjected to electrophoresis on 17.5% SDS-polyacrylamide gels. Autoradiographs of these gels were scanned for the repressor band and the peaks cut out and weighed. The amount of repressor in the control was multiplied by dilution factors to determine the amount of repressor in each binding mix. As an equal amount of the repressor preparation was used for each binding mixture, an average of the amount of repressor in the controls was used to normalise the amount of repressor in fractions of the gradients. The per cent of the total repressor recovered in fractions of gradients with a, repressor alone ($\bullet$); b, repressor plus plasmid 25 DNA ($\times$); and c, repressor plus pMB9 DNA (Δ) is shown. The vertical arrows indicate the position of DNA in the gradients. DNA of both plasmid 25 and pMB9 was digested with the restriction enzyme *Hind*III (Bethesda Research Labs) which cleaves both DNAs once, making linear molecules. Uncut plasmid 25 also shows binding of the repressor; however, two peaks of bound repressor are detected, corresponding to DNA in the monomer and dimer covalently closed circular forms. In these conditions the dimer DNA sediments further down the gradient than the monomer form. Plasmid 25 and pMB9 were purified from strain χ 1274 (*E. coli* K12, Thi^- , Thr^- , Leu^- , LacY^- , MalA^- , MtL^- , Xyl^- , Ara^- , Gal^- , Str A , ton A , λ^R , azi^- , Min^- , su^+) according to the procedure of Clewell¹³.

repressor found in the top fraction of the gradients did not decrease significantly with increasing amounts of DNA (Table 2). The amount of inactive repressor in any one preparation increased with the storage time of minicell preparations before use.

As shown in Table 2, both the amount of DNA-bound repressor and the overall amount of repressor recovered increase with increasing concentrations of plasmid 25 DNA, except at very high concentrations of DNA. Similarly, more repressor was recovered when pMB9 DNA was added to the same preparation, than was recovered after sedimenting the preparation alone. In this case, however, the repressor was not associated with pMB9 DNA, but was spread throughout the gradient (Fig. 2). This suggests that the active repressor proteins will bind to each other, if not bound to DNA, forming aggregates which sediment rapidly in the gradient. Thus, if the repressor extract is sedimented without added DNA, much of the active repressor goes to the bottom of the gradient.

This repressor-repressor interaction must be weaker than repressor-DNA binding, as any DNA added to the repressor seems to inhibit aggregate formation. As the repressor dissociates from non-operator-containing DNA (that is, pMB9)

during sedimentation, however, it will begin to form repressor-repressor aggregates, and this repressor is detected throughout the gradient. Repressor binding to operator-containing DNA (plasmid 25 DNA) is tight, so that very little repressor dissociates from the DNA during sedimentation and no aggregation of the repressor occurs. This finding is consistent with the observation that there is a decrease in the amount of total repressor recovered in binding assays carried out at 0.3 M rather than 0.1 M NaCl. The decrease in recovery is more dramatic with D108 or pMB9 DNA than with Mu or plasmid 25 DNA.

Further characterisation of the nature of the binding of Mu repressor to Mu DNA (and repressor-repressor interaction) requires purification of significant amounts of repressor protein. This is feasible because large quantities of repressor can be made in strains carrying plasmid 25 (or similar recombinant plasmids), and crude DNA binding assays (like that described above) allow easy detection of active repressor during purification.

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Surface and inside volumes in globular proteins

MEASUREMENTS of the surface area accessible to solvent provide a convenient definition of the surface and the inside volumes in proteins of known X-ray structure. The study of the accessibility to solvent of amino acid residues in several proteins¹⁻³ has confirmed the early observation that polar residues are found mostly on the surface and non-polar residues mostly inside globular protein structures. But the accessibility shows systematic variations with the molecular weight, because of the change in surface to volume ratio. Experimental data⁴ indicate that the accessible surface area A (in $\text{\AA}^2$) of monomeric globular proteins follows the law^{5,6}:

$$A = 11.1 M^{2/3} \quad (1)$$

which implies that the mean accessible surface area per residues decreases like $M^{-1/3}$ (where M is molecular weight) with increasing M , from about $68 \text{ \AA}^2$ for proteins of 6,000 molecular weight to $38 \text{ \AA}^2$ for proteins of 35,000 molecular weight. Thus, the accessibility to solvent is not a characteristic of the amino acids.

We made histograms of the accessible surface areas of residues in a sample of 22 proteins, and found that the number n_B of buried residues, defined as having less than a given accessible surface area A_m , varies with the total number n of residues in the

polypeptide chain according to:

$$n^{1/3} - n_B^{1/3} = k \quad (2)$$

where k depends only on A_m . Completely buried residues (zero accessible surface area) are few: 4 in pancreatic trypsin inhibitor (58 residues), 40 in carboxypeptidase A (307 residues). Better statistics are obtained by choosing $A_m = 10$ to $20 \text{ \AA}^2$, which amounts to consider as buried residues which have one or two atoms in contact with the solvent. With $A_m = 20 \text{ \AA}^2$, the average value and standard deviation of k are 1.62 ± 0.13 . The geometrical significance of equation (2) is apparent in Fig. 1: k represents the thickness of the surface layer made of residues in contact with the solvent. While the hard sphere model of globular proteins, first presented by Fisher⁷, is in formal agreement with equations (1) and (2), the fit only implies that the gross geometrical properties of globular proteins (surface and volume) are those of a set of solid bodies which deviate in similar ways from a spherical shape. This is not true of subunits in oligomeric proteins: the subunits of tetrameric lactate dehydrogenase⁴ and glyceraldehyde-3-phosphate dehydrogenase have much larger accessible surface areas and fewer buried residues than expected from equations (1) and (2).

Table 1 gives the amino acid composition of our sample of buried and accessible residues. The buried residues make up the protein inside; 56% are Leu, Ile, Val, Ala, or Gly, another 22% are Phe, Ser, Thr or Cys, and 22% are of the 11 other types. The protein surface has a much more regular amino acid composition than the inside: non-polar residues are by no means absent, even though Asn, Asp, Glu, Gln, Lys and Arg constitute 40% of the accessible residues. The ratio f of the molar fractions of each amino acid in the two samples is of particular interest. It

Table 1 Amino acid composition of the inside and surface

Residue	Molar fraction		Free energy (kcal mol ⁻¹)		
	Buried	Accessible	f	ΔG_i	ΔG_h
Leu	11.7	4.8	2.4	0.5	1.8
Val	12.9	4.5	2.9	0.6	1.5
Ile	8.6	2.8	3.1	0.7	
Phe	5.1	2.4	2.2	0.5	2.5
Cys	4.1	0.9	4.6	0.9	
Met	1.9	1.0	1.9	0.4	1.3
Ala	11.2	6.6	1.7	0.3	0.5
Gly	11.8	6.7	1.8	0.3	0.0
Trp	2.2	1.4	1.6	0.3	3.4
Ser	8.0	9.4	0.8	-0.1	-0.3
Thr	4.9	7.0	0.7	-0.2	0.4
His	2.0	2.5	0.8	-0.1	0.5
Tyr	2.6	5.1	0.5	-0.4	2.3
Pro	2.7	4.8	0.6	-0.3	
Asn	2.9	6.7	0.4	-0.5	
Asp	2.9	7.7	0.4	-0.6	
Gln	1.6	5.2	0.3	-0.7	
Glu	1.8	5.7	0.3	-0.7	
Arg	0.5	4.5	0.1	-1.4	
Lys	0.5	10.3	0.05	-1.8	

Molar fractions (%) of the 20 amino acids in samples of 2,001 buried residues (less than $20 \text{ \AA}^2$ accessible surface area) and 3,220 accessible residues (more than $20 \text{ \AA}^2$); f is the ratio of the buried/accessible molar fractions; $\Delta G_i = RT \ln f$; ΔG_h is the free energy of transfer from an organic solvent to water⁸. Cys residues with a free -SH group are few in the sample; our data are more representative of substituted -SH groups (disulphides, metal adducts or thioethers).

represents the partition coefficient of this amino acid between the surface and inside volumes. Consequently, $\Delta G_i = RT \ln f$ may be taken as the free energy of transfer from the inside to the surface. Though the exact value of f depends on the choice of A_m made in defining buried residues, the effect is small: reducing A_m from $20 \text{ \AA}^2$ to $10 \text{ \AA}^2$ changes the free energies by no more than their statistical standard deviation of $0.1\text{--}0.2 \text{ kcal mol}^{-1}$. Thus f and ΔG_i are characteristic of the amino acids in globular proteins.

In a protein of n residues, the most probable fraction g of buried residues of one type, say valine, is easily calculated from the data of Table 1:

$$g = \frac{fn_B}{n - n_B + fn_B} \quad (3)$$

where n_B is given by equation (2). Thus, the fraction of valines which are buried increases from 0.4 to 0.7 when the chain length increases from 60 to 300 residues, while it remains less than 0.1 for lysines.

Chothia³ has noted that the average accessibility of residues in proteins is not correlated to the free energies ΔG_h of transfer from an organic solvent to water, measured by Nozaki and Tanford⁸. This is seen clearly in Table 1: ΔG_i is governed by the presence or absence of oxygen and nitrogen atoms in the side chain^{1,3}. These polar atoms must find hydrogen bond partners when the residue is buried, a stringent requirement except for Ser and Thr residues, the hydroxyl group of which can easily form a hydrogen bond to adjacent main chain carboxyl groups⁹. Proline appears as polar; its imino nitrogen cannot participate in normal main chain hydrogen bonds and behaves as a part of the side chain.

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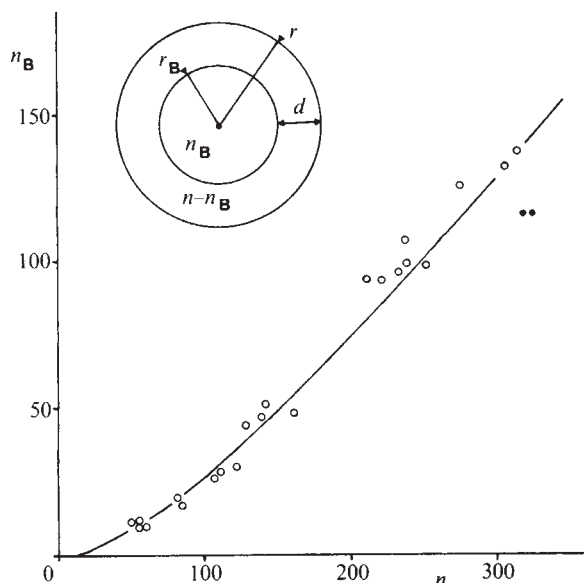


Fig. 1 Buried residues in globular proteins. The accessible surface areas of individual residues are calculated according to Lee and Richards¹ using a computer program of M. Levitt. The atomic coordinates are obtained from the Protein Data Bank, Cambridge, UK. The following 22 protein structures are used here and in Table 1: insulin, ferredoxin, rubredoxin, pancreatic trypsin inhibitor, high potential iron protein, cytochrome b_5 , parvalbumin, cytochrome c_2 , ribonuclease S, hen lysozyme, staphylococcus nuclease, flavodoxin, phage T4 lysozyme, papain, trypsin, concanavalin A, elastase, α -chymotrypsin, carbonic anhydrase B, subtilisin, carboxypeptidase A, thermolysin. The number n_B of residues with less than $A_m = 20 \text{ \AA}^2$ of accessible surface area is plotted against the total number n of residues in the polypeptide chain (open circles). Filled circles represent the subunits of lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. The insert represents the protein as made of two concentric spheres, the volumes of which are proportional to n and n_B ; the radii are proportional to $n^{1/3}$ and $n_B^{1/3}$, and therefore k is proportional to the thickness d of the surface layer, made of $n - n_B$ accessible residues. For the 22 proteins: $A_m = 0 \text{ \AA}^2$, $k = 3.0 \pm 0.4$ (s.d.), $d = 9.6 \text{ \AA}$; $A_m = 10 \text{ \AA}^2$, $k = 2.0 \pm 0.2$, $d = 6.4 \text{ \AA}$; $A_m = 20 \text{ \AA}^2$, $k = 1.62 \pm 0.13$, $d = 5.2 \text{ \AA}$. The solid line is calculated from equation (2) with $k = 1.62$.

Dimeric association of band 3 in the erythrocyte membrane demonstrated by protein diffusion measurements

BAND 3, a major constituent of the human erythrocyte membrane, comprises a class of membrane-spanning proteins which are implicated in anion and possibly other transport functions¹⁻⁴. Various observations indicate that band 3 proteins may exist as dimers in the membrane⁵⁻⁹. The most direct indication has been obtained from chemical cross-linking experiments. The band 3 dimer is an early product of various cross-linking reactions⁷, and the particular case of cross-linking by oxidative disulphide bridge formation results in almost quantitative dimerisation of band 3 (ref. 8). However, cross-linking experiments are in general inconclusive for they do not indicate whether a successful cross-linkage reflects a naturally occurring stable complex, or is the result of collisions between independent proteins which rapidly diffuse over small distances. In the recent elegant studies of Kiehm and Ji⁹, rapid photochemical cross-linking was achieved by photolysing with a millisecond flash. Whilst these experiments undoubtedly provide the best evidence so far obtained for the existence of the band 3 dimer, they still leave some doubts concerning the possibility of collision-induced crosslinks. These can only be ruled out if the mean time between collisions is shown to be much longer than the lifetime of the flash-induced reactive species. The latter time was assumed to be short (of the order of 1 ms) on the basis of solution studies but was not measured in the experimental conditions. The collision frequency is a function of the local lateral diffusion coefficient. Band 3 diffusion over distances $>1 \mu\text{m}$ seems to be slow in the erythrocyte membrane^{10,11}, but as discussed elsewhere¹², this may reflect restrictions to long range diffusion and does not rule out the possibility of more rapid local diffusion. If the local diffusion coefficient is in the range 10^{-9} to $10^{-10} \text{ cm}^2 \text{ s}^{-1}$, the mean time between collisions is also of the order of 1 ms (ref. 9). A lateral diffusion coefficient in this range is consistent with the rotational diffusion of band 3 (ref. 12). Here we present a new physical approach to determining the self-association of band 3, based on the measurement of rotational diffusion. The rotational diffusion coefficient of an intrinsic membrane protein is strongly dependent on the diameter of its cross-section in the plane of the membrane. We show that cross-linking band 3 into dimers produces no observable decrease in the rotational diffusion coefficient of band 3 in the membrane. We therefore conclude that the dimer pre-exists in the membrane and is not a product of the cross-linking reaction following random collisions.

In the present experiments, the rotation of band 3 was measured by observing the flash-induced transient dichroism of a triplet probe, as previously described^{12,13}. However, band 3 was labelled with eosin maleimide instead of the eosin isothiocyanate previously used; essentially similar results are obtained with either probe. Dimerisation of band 3 in the membrane was achieved by copper-phenanthroline-catalysed oxidation of cytoplasmic sulphhydryl residues to form disulphide bridges, as described by Steck⁸.

Figure 1 shows the result of the cross-linking reaction as visualised by SDS-polyacrylamide gel electrophoresis. Band 3 has disappeared from its normal position in the gel, moving to a new band with a molecular weight of approximately 180,000, corresponding to the band 3 dimer. This result is identical to that reported by Steck⁸.

The rotational mobility of band 3 in the cross-linked membranes was measured and compared with that of untreated controls. The transient dichroism measurements were analysed

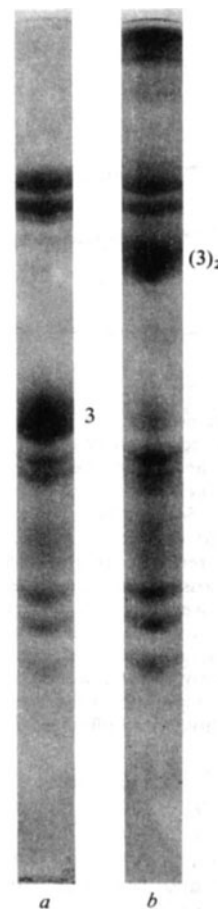


Fig. 1 Dimerisation of band 3 by oxidative disulphide bridge formation. Intact human erythrocytes were labelled with eosin-5-maleimide as described previously for other eosin derivatives^{12,13,19}, except that incubation was for 1 h at room temperature. These conditions yield approximately 1 molecule of bound eosin per band 3 monomer. Ghosts were prepared by hypotonic lysis¹² and given a final wash in 5 mM sodium phosphate, pH 8.0. They were allowed to react for 1 h at room temperature with one volume of *o*-phenanthroline (200 μM) and copper sulphate (40 μM) in the same buffer. The reaction was ended by the addition of 30 volumes 1 mM EDTA and 5 mM sodium phosphate, pH 8.0, and by a further wash in 5 mM sodium phosphate, pH 7.4. The control sample was subjected to the same procedures but without the addition of the copper-phenanthroline reagent. Ghosts were then dissolved for electrophoresis and run on cylindrical 4% polyacrylamide gels in 0.2% SDS as described by Steck⁸. 3 and (3)₂ denote the positions of the band 3 monomer and dimer, respectively. Gel a, control; gel b, cross-linked ghosts.

by calculating the absorption anisotropy $r(t)$ given by

$$r(t) = \frac{A_{\parallel}(t) - A_{\perp}(t)}{A_{\parallel}(t) + 2A_{\perp}(t)} \quad (1)$$

where $A_{\parallel}(t)$ and $A_{\perp}(t)$ are, respectively, the absorbance changes at time t after the flash for light polarised parallel and perpendicular with respect to the polarisation of the exciting flash. Figure 2 shows the time dependence of r for dimerised and untreated band 3. It is clear that the shapes of the two curves are virtually identical.

As discussed elsewhere^{12,14}, the predicted form of $r(t)$ for a protein which rotates only about the axis normal to the plane of the membrane is

$$r(t) = A_1 \exp(-D_{\parallel}t) + A_2 \exp(-4D_{\parallel}t) + A_3 \quad (2)$$

where $D_{\parallel}$ is the rotational diffusion coefficient and A_1, A_2, A_3 are constants. To make a quantitative comparison of the cross-linked and control samples, we made a least-mean-square fit of

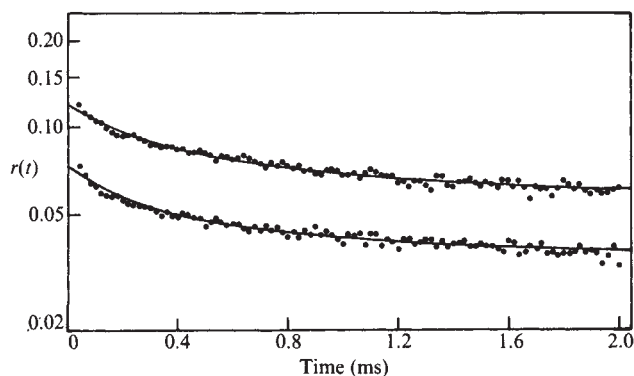


Fig. 2 Time dependence of the absorption anisotropy for untreated and cross-linked erythrocyte membranes. Flash-induced transient dichroism of the eosin probe was measured using a flash photolysis apparatus which is described in detail elsewhere¹³. The sample was excited at 540 nm by a linearly polarised flash of duration 1–2 μ s. Absorbance changes arising from ground state depletion were measured at 520 nm. Upper curve, untreated control; lower curve, cross-linked as described in the legend to Fig. 1. Experimental points were obtained by averaging 32 signals. The solid lines were obtained by fitting the data to equation (2). For illustrative purposes the two curves have been artificially separated by a vertical displacement of the lower curve by 20%. Otherwise the data almost exactly superimpose. Measurements were made at 37 °C in 5 mM phosphate buffer, pH 7.4, under argon.

the data to equation (2). In this way we obtain $D_{\parallel} = 1,296 \pm 99 \text{ s}^{-1}$ and $1,492 \pm 202 \text{ s}^{-1}$ for the control and cross-linked membranes, respectively.

If we regard band 3 as a cylinder of radius a immersed to a depth h in the membrane, then the coefficient for rotational diffusion about the membrane normal is given by¹⁵

$$D_{\parallel} = (kT)/(4\pi a^2 h \eta) \quad (3)$$

where η is the membrane viscosity. Thus, the rotational diffusion coefficient depends on the square of the radius of the rotating species. Assuming that dimerisation increases the radius of the particle by a factor of 1.5–2, we thus expect a two- to fourfold difference in $D_{\parallel}$ between the monomer and the dimer. As we fail to observe any decrease in $D_{\parallel}$ following cross-linking, we conclude that the band 3 dimer exists as a stable complex in the erythrocyte membrane independent of chemical cross-linking.

A possible objection to this conclusion is that the cytoplasmic portion of band 3 on which the reactive SH-group is located¹⁶, is attached by a flexible linkage to the major part of the protein whose rotation we observe. Hence, cross-linking in this position might not change the rotation of band 3 monomers. However, Ross and McConnell have reported¹⁷ that a spin label bound to the same SH-group is 'immobilised' (on the conventional electron spin resonance time scale). This strongly suggests that the cytoplasmic fragment is rigidly attached to the bulk of the protein and is not an independently rotating entity.

It should also be pointed out that cross-sectional shapes of band 3 could in principle be devised which would considerably reduce the expected change in $D_{\parallel}$ on association of monomers into dimers. Because we could detect a change in radius with cross-linking as small as 15%, we consider this a rather improbable explanation of our data. Taken together with the earlier chemical studies, there can be little doubt that band 3 does exist as a stable dimer within the human erythrocyte ghost membrane. However, further association of band 3 dimers among themselves or with other integral membrane proteins such as the major sialoglycoprotein are not ruled out.

The functional significance of the band 3 dimer is unclear. As the major part of band 3 is the anion transport system^{1,2,18}, it is tempting to speculate that the anion channel is formed at the interface between the two subunits of the dimer. However, inhibition studies with disulphonic acid stilbene inhibitors¹⁸ and

eosin maleimide¹⁹ show that about one inhibitor bound per band 3 monomer is required for total inhibition of anion transport. This suggests that there are two anion binding sites per channel, if there is only one channel per band 3 dimer. Alternatively, one half of the inhibitor molecules could be bound to nonspecific sites on the protein.

Thus, the experiments reported here demonstrate how rotational diffusion measurements can be used to detect stable oligomeric complexes of proteins within biological membranes. We anticipate that the approach may be extended to other membrane proteins, for example, the $(\text{Mg}^{2+}, \text{Ca}^{2+})\text{ATPase}$ of the sarcoplasmic reticulum, where the possibility of self-association is now under consideration^{20,21}.

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Errata

In the review article 'Multiple receptors for dopamine' by J. W. Keibarian and D. B. Calne, *Nature* **277**, 93–96, line 27 in the right-hand column of page 95 should read 'activity, have been used to...' and line 34 should read 'studies are only just beginning to be...'

In the letter 'MHC matching shows that at least two T-cell subsets determine resistance to HSV', by E. L. Howes *et al.*, *Nature* **277**, 67–68, in line 10 in paragraph 6, for 'in the thymus' read 'in the remnant thymus'.

In the letter 'Loss of immunoreactivity in long-term bone marrow cultures' by T. M. Dexter and E. Spooner, *Nature* **275**, 135–136 (1978), on page 136, lines 5 and 8 for H-v-G read G-v-H.

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matters arising

Coesite and stishovite in the Vredefort, South Africa

RECENTLY I have been involved in tectonophysical research in the Witwatersrand collar sequence of the Vredefort dome, concentrating on microfabric studies in the collar quartzites with particular reference to the shock metamorphic microstructures found in some of these rocks. These shock microstructures include planar features, crystallographically controlled cleavage, crystallographically controlled faults and mosaic extinction. Other microstructures include extreme undulose extinction and deformation bands.

Planar features are the most widely accepted indicators of shock deformation and in the Vredefort collar quartzites they occur as decorated, planar discontinuities parallel to crystallographic planes in the quartz. The crystallographic orientation of planar features can be used to estimate the magnitude of shock pressures sustained by the rock sample¹⁻³, and has been used with considerable success at the Charlevoix and Slate Islands structures^{2,3}. The technique involves measuring the orientation of planar features in 20 to 30 grains in a sample, classifying each grain on the basis of its planar feature content¹, and assigning to each a pressure value calculated from experimental data^{2,3}. The shock pressure in the rock is then calculated as the mean of shock pressures in individual grains.

Samples from the Witwatersrand collar (Fig. 1) have been used in the type of analysis described above. Shock estimates range from 59 to 148 kbar. A diagram showing estimated shock pressure as a function of distance from the core-collar contact is shown in Fig. 2 in which the

curve of best fit is nonlinear and given by

$$P_{sh} = 145.47D^{-0.39}$$

where P_{sh} is the estimated shock pressure and D is the distance from the core-collar contact. There is a strongly evident decrease in shock pressure away from the contact.

Many of the shock microstructures in the Lower Witwatersrand quartzites, however, have been recovered and recrystallised. The amount of recrystallisation increases towards the core, from minor recrystallisation away from the core (in which minor amounts of new quartz occur along planar features and grain boundaries) through major recrystallisation (where a large percentage of the rock is recrystallised with only relict primary grains), so that the Hospital Hill quartzites are completely recrystallised. Minor recrystallisation extends to the outermost quartzites of the collar. Major recrystallisation, however, occurs in a zone⁴ which has the same shape as, but is not as extensive as, the limit of contact metamorphism in the collar (which is shown in Fig. 1), so that it is probable that recrystallisation in the quartzites is a result of the thermal metamorphism which produced the contact metamorphic metapelites in the collar.

Stöfler⁵ has demonstrated that after their formation, coesite and stishovite are metastable and susceptible to reversion during thermal metamorphism. Certainly one would expect that large amounts of high pressure quartz polymorphs would be found in the lowermost Witwatersrand rocks (those closest to the core) since this is where the shock pressures are greatest (Fig. 2). However, as Martini has shown, none are found; samples from the Hospital Hill quartzite examined by Martini (those from Marra 395, Fig. 1 and Vlak-

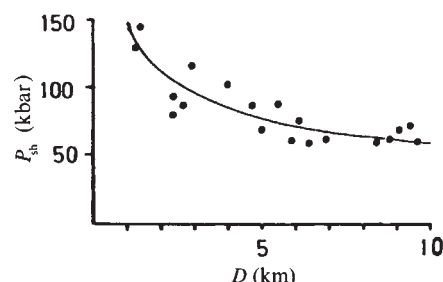


Fig. 2 A plot of estimated shock pressure (P_{sh}) against distance from the core-collar contact (D) for samples from the Vredefort collar sequence.

spruit 1180, which is off Fig. 1 to the south-west) are barren. However, Martini did find coesite and stishovite at Weltevrede 167 and coesite at Ospan 238 which are high up in the Witwatersrand sequence (Fig. 1).

I suggest, therefore, that no polymorphs are found in the lowermost collar rocks because they were affected by the post-shock, metamorphic overprint. Only those rocks not strongly affected by metamorphism and/or recrystallisation (that is, the rocks further from the core) would retain their high pressure polymorphs of quartz.

The sample which Martini took on Nooitgedacht 508 (Fig. 1) is, however, barren. This is well outside the thermal aureole and does not fit comfortably into the model outlined above. My sample from Nooitgedacht 508 (sample 9, Fig. 1) shows evidence of minor amounts of recrystallisation along planar features; this indicates that these rocks were very slightly affected by the metamorphism. This may account for the lack of high pressure minerals in these rocks.

This work was supported by the CSIR and the South African National Geodynamics Project.

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Fig. 1 A simplified geological map of the Vredefort dome (modified after Bisschoff⁶) showing the limit of metamorphism in the collar (LCM) and the localities of samples used in the shock pressure analysis (dots). The approximate localities of farms on which Martini⁷ took samples are shown as triangles where W is Weltevrede 167, O is Ospan 238, M is Marra 395 and N is Nooitgedacht 508. The towns of Parys (P) and Vredefort (Vr) are also shown.

MARTINI REPLIES—The microfabric investigations conducted by Lilly are of great interest as they not only confirm the very high pressure conditions having

characterised the Vredefort Dome event, but also give a geographical distribution of these conditions. However, the number of samples analysed for coesite and stishovite is statistically not representative and at this stage it is not possible to demonstrate any clear field relationship between the distribution of high-pressure silica polymorphs and the extent of Lilly's metamorphic zone. On theoretical grounds, I agree that metamorphism must account at least in part for the absence of these minerals, but a much more detailed investigation is needed to clarify this aspect. Other factors may be also responsible for the absence of coesite and stishovite.

The post-shock metamorphism described by Lilly results probably from the high temperature of the rocks immediately before impact. Due to the great magnitude of this event, deep portions of the continental crust must have been shattered, at depths where temperatures attained several hundred degrees. In the Vredefort Dome these deep portions are represented by the lower part of the Witwatersrand Supergroup and by the granitic core, that is those rocks in which the post-shock metamorphism is developed.

After the quick uplift which occurred immediately after impact, rocks brought

very close to the surface cooled down rapidly. This part is now eroded. In contrast, rocks which remained at a depth of several hundred metres or more, which presumably constitute the present-day outcrops, remained warm long enough to allow for the recrystallisation of shock metamorphism textures and reversal of coesite and stishovite into quartz. This probably explains the post-shock metamorphism, which in fact has been initiated before the impact.

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Expected shapes of asteroids

FUJIWARA *et al.*¹ have demonstrated experimentally that random breakage of basalt blocks to simulate asteroid formation produces a predominance of what the sedimentary petrologists call blade-shaped fragments; rod-like and sphere-like fragments tended not to be produced. These results agree well with a theoretical analysis² of the random breakage situation in which very simple probabilistic

methods were used to estimate the formation of the four basic Zingg shapes (disks, spheres, blades and rods). It was proposed that, with an attainable 10% accuracy of dimensional resolution, random breakage of an isotropic rock material would yield 72% blade shapes, 27% tetragonal shapes (rods and disks) and 1% equiaxed shapes (spheres). The sphere value agrees well with the Fujiwara *et al.* results but the theoretical estimation did not allow rods and disks to be separated. It is possible that the experiments¹ produced several disk particles which were judged to be blades. In some earlier experiments Drake³ examined various rock types; basalts and other aphanitic rocks produced approximately the same number of spheres as predicted by the theory but the number of blades was greater, at the expense of rods and disks; as Fujiwara *et al.* have also found.

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reviews

Major environmental controversy

B. A. Thrush

The Ozone War. By L. Dotto and H. Schiff. Pp. 342. (Doubleday: New York, 1978.) \$10.

THE most interesting environmental controversy in recent years has surrounded the potential effects of supersonic aircraft and of chlorofluoromethanes (CFMs) on the stratospheric ozone which protects the Earth's surface from harmful ultraviolet light. Although the basic mechanism by which oxygen is converted into ozone had been propounded in 1930 by Chapman, the atmosphere had become so much a preserve of meteorologists and atmospheric physicists that, forty years later when it was suggested that the nitrogen oxides in the exhausts from supersonic aircraft would deplete the ozone layer, no-one knew what the natural amount's of nitrogen oxides in the ozone layer were.

In such circumstances, it is hardly surprising that projected fleets of 500 supersonic planes were predicted to produce large depletions of ozone, and that the major Climatic Impact Assessment Program (CIAP) was established in the United States. When CIAP's report appeared in 1975, the problem had largely disappeared because of the paucity of supersonic aircraft and better knowledge of the problem. By now, Molina and Rowland's paper (*Nature*, **249**, 810; 1974) had switched attention to the CFMs, particularly F-11 (CFCl₃) and F-12 (CF₂Cl₂) which are extensively used as aerosol propellants, refrigerants and foam-blowing agents. The great stability of these compounds ensures that they are eventually transported up to the stratosphere where their decomposition products will remove ozone. Because the predicted ozone depletions increase slowly to a maximum over a period of 50–100 yr, they would be very hard to detect against the short-term fluctuations in ozone cover; the argument therefore revolved around modelling calculations. To the thriving and vocal industries involved, 'theory' and 'hypothesis' rapidly became pejorative words, although research was soon financed. US Government agencies battled briefly for the 'lead' role. Many scientists joined the bandwagon and the Congressional Record sometimes provided rapid publication for unrefereed work. The public were often confused by contradictory news stories, and (although it is customary to blame

the press for seeking scare stories) some scientists provided them willingly, others unwittingly through the difficulty of explaining a complex problem clearly.

This book gives a fascinating insight into the people and organisations who became involved in these controversies. To those accustomed to the more phlegmatic debate of such issues in this country *The Ozone War* may sound an exaggerated title, but many of the participants saw themselves as crusaders against the lack of action or the over-reaction by Environmentalists, Industry or Government Agencies, with Congressional Hearings providing a natural sounding board.

The debate calmed down when the National Academy of Sciences (for once not described as 'prestigious' in a North American publication) appointed a panel to examine the CFM problem. Their report in 1976 concluded that release of F-11 and F-12 at the 1973 rates would eventually produce an ozone depletion of 7% with uncertainty limits from 2–20%. Predictably, some saw the upper limit as a cause for immediate action and others saw the lower limit as a reason for delaying action. Surprisingly, the latter have taken heart from recent discoveries which have reduced estimates of ozone depletion by supersonic aircraft and by increased use of nitrogenous fertilizers, despite the concomitant increases in the predicted effects of CFMs.

Surprisingly, there has been no debate on what would be an acceptable ozone depletion, and the potential effects of ozone depletion have received much less attention. A 10% decrease in ozone would increase damaging ultraviolet light by about 20%, probably causing a similar increase in skin cancer. However, such diseases are only responsible for about 1% of cancer deaths, although the figure is increasing rapidly because of the

fashion for suntans in recent years. Is excessive sunbathing a health hazard or should we be more concerned about the considerable effect of increased ultraviolet light on phytoplankton? Will ozone depletion affect atmospheric circulation and therefore the climate? Here the answer is that it is probably less than that due to the increased carbon dioxide from burning fossil fuels.

Basically, the aerosol spray provides an easy target for the environmentalist, whereas the other uses of F-11 and F-12 in air conditioners, in insulating foam and in refrigerators require a more careful cost-benefit analysis. Another interesting point to be considered is how far different nations can agree about what is a global problem, as the effects of these compounds are quite independent of where on the Earth's surface they are released.

The history of how the vital processes in atmospheric chemistry were discovered and the rush to join the bandwagon are reminiscent of *The Double Helix* but with a much larger cast and broader setting in which Industry, Government Agencies and individual scientists strive to protect their own interests. The principal events and characters are well described and although one protagonist may not be pleased to see the first measurement of the rate of an important reaction attributed to another, the authors are to be congratulated on producing a highly readable and accurate account of how a major environmental controversy evolved in the United States. There are lessons here for all, particularly for those who wish to know how newspapers can come to publish contradictory or dogmatic reports about an unresolved issue. □

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Plant-parasitic nematodes

Ecology of Plant-Parasitic Nematodes. By D. C. Norton. Pp. 268. (Wiley: New York and Chichester, UK, 1978.) £16.95.

THIS book is an introductory account of plant-parasitic nematodes in relation to their environment. It is not an updated version of Wallace's book

Nematode Ecology and Plant Disease, as the author tackles the subject from, what he terms, a broad ecological viewpoint. Emphasis is placed on the effects of the environment rather than on the disease aspect, and discussions on nematode damage to plants are largely omitted.

The first five chapters cover aspects

of nematode distribution, dispersal, habitats, groupings and communities, and population change. These include some interesting, but all too brief, discussions on the origins and recent past of nematodes, clustering, their means of dissemination, and some methods of analysing nematode communities and populations. It is well accepted that parasitic nematodes are always found associated with all forms of plant life; and, under the heading of habitats, the author mentions their associations with fungi, algae, lichens, mosses and higher plants. An introduction to population changes is presented in which seasonal changes, growth patterns, r and k selection, and sex ratios are discussed.

In the following two chapters the author deals with the soil environment: in the first of these, he discusses basic soil principles referring frequently to the interrelationships between soil factors; and in the second, he discusses the effects of physical and chemical soil changes on the nematodes, the section on effects of soil fertility is of particular interest to plant nematologists. The remainder of the book is concerned with survival, biological control, and interactions between nematodes and with other organisms. The final chapter is a disappointingly short account of the effects of agricultural practices on

nematode populations, although the author acknowledges that the influence of cultural practices on nematodes has not received the attention it deserves.

There are errors throughout the book which could have been avoided—for example, the use of some defunct nematode nomenclature. Dr Norton has summarised much of the relevant literature in tabular form rather than discussing it in the text; even so these lists are not exhaustive. Most of the literature cited and the majority of the discussions relate to the ecology of nematodes in the United States. Although these are often relevant to work elsewhere, the book seems to have been written with the North American market in mind.

It is a useful introductory book to plant nematode ecology in which the author has brought together information from ecological disciplines relating to nematodes under one cover. Some interesting ideas are introduced, but it is not a sufficiently in-depth, critical or comprehensive study of the subject to entirely satisfy the needs of the professional nematologist. **John Bridge**

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Search for psychological reality

Linguistic Theory and Psychological Reality. By M. Halle, J. Bresnan and G. A. Miller. Pp.329. (MIT Press: Cambridge, Massachusetts, and London, 1978.) £12.25; \$17.50.

THE search for psychological reality is on again. Last time the psychologists did the searching with the new toys they did not understand; this time it is the linguists (at least in approach) who are doing the searching and get the reviewer very confused in the process. It is a book without Chomsky, but with his bust on the mantelpiece, in which we start with the acceptance that linguistics is a branch of psychology but come away with the feeling that they mean that psychology is a branch of linguistics. Linguists and psychologists talk together; they write chapters which refer to each other (good editorial work here) but to scarcely anyone else.

We start with a chapter by Bresnan on "A realistic transformational grammar". The largest part of this is devoted to a beautiful linguistic demonstration of how some facts which used to be handled by transformations

are now handled in the lexicon. The linguistics is generally judged among the best being produced currently and is very convincing. The 'realistic' in the title is introduced early. She makes the explicit claim that if a grammar is "to represent the knowledge of the language user in any psychologically interesting way" then all the grammatical rules must be realisable in a psychological model. If this is a serious claim then one might think that the best strategy would be to focus first on the types of constraint that psychology might impose. Instead we get the impression that the psychology is an afterthought. A hand reaches out languidly to pluck out a single piece of supporting data or intuition but no more. Thus Bresnan gives us reasons for arriving at passive and short passive forms directly instead of by means of transformations. At the end she notes that one of the other contributors to the volume reports that short passives (for example, the dog was bitten) was acquired before full passives by children. The tone of voice is almost congratulatory—how sensible of the children to realise the correct derivation! Such use of psychological evidence permeates the book.

Wanner and Maratsos give an example of a different kind of model, an Augmented Transition Network

(ATN). One of their aims is to parse sentences without recourse to transformations. Strangely, then, we are also told several times that these parsers "are not incompatible with a transformational model of language". Remembering that Bresnan still found transformations necessary, this is reassuring. However, if Bresnan's grammar is psychologically realistic and the ATN's have psychological reality doesn't the latter have to be the realisation of the former?

The lexical side of language is no better handled except in a little piece on "The child as a word learner" by Susan Carey. In this she exposes the magnitude of the task of pairing thousands of words with their meanings in a few years. No answers, but a hint of the psychological questions. George Miller has a chapter on the semantic relations among words. This is a development of the ideas he put forward with Johnson-Laird in *Language and Perception* (reviewed in *Nature*; 264, 124, 1976). Miller makes some tight formal points but his thinking doesn't make as close a contact with psychological issues as the earlier work. Still, this is better than Jackendoff who, like Miller, wants to claim that semantics must derive from a general theory of conceptual structures. This seems a saner view of universality than one which bases it exclusively on language, but Jackendoff effectively turns the relationship around by using linguistic analysis to derive the semantic system from which the cognitive universals should emerge. The effectiveness of his argument may be judged from a non-linguistic parallel which he describes—"the ability of a dancer to create appropriate dances for given music . . . in such a way that there is substantial interpersonal agreement on how appropriate the translation is." There must be generalisations which mediate the "complex and subtle" expression "determined at least in part along lines laid down by the nature of the organism." (Q.E.D.).

This kind of nativism gets another airing by Halle in a discussion of phonology where a 1971 result of Eimas is cited in support of innate mechanisms of speech analysis. Half-a-dozen or more recent refutations of this view have apparently gone unremarked (see, for example a chapter by Mehler in *Psycholinguistics*, series 2, ed. Morton and Marshall; Elek, 1979). There is a lot of intelligence in this book overall and a review by a linguist might give a much more positive impression but the *and* in the title is disjunctive.

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Haematological overkill

The Year in Hematology, 1978. Edited by R. Silber, J. LoBue and A. S. Gordon. Pp. 517. (Plenum Medical: New York and London, 1978.)

ALL the signs are that we are entering a state of literary polycythaemia. For non-clinical readers this means that current scientific writing is suffering from a pathological excess of blood. As well as the increasing number of textbooks and monographs on the subject and a rapidly proliferating series of haematology journals, many of which carry review articles, there are regular review series such as *Clinics in . . .*, *Recent Advances in . . .*, *Topics in . . .*, *Seminars in . . .*, *Progress in . . .*, and many more, all offering minor variations on the same theme. Added to all this the general scientific and medical journals are giving a considerable amount of space to articles on haematological topics, and nearly every international meeting on the subject now insists that authors prepare manuscripts for a published account of the proceedings. Presumably somebody reads all these reviews and the publishing houses and the Inland (and Internal) Revenues (if not the authors) are waxing rich. However, as blood letting rather than transfusion is the more logical approach to the treatment of polycythaemia, surely the appearance of yet another review series in this field must raise serious questions about the value of all this repetitive writing.

The Year in Hematology first appeared in 1977 and on the jacket of the 1978 edition the series is described as offering "a comprehensive overview of the latest findings and trends that have occurred during the last year, critically appraising the most recent observations of normal and abnormal function in the hemopoietic system". A glance at the list of references at the end of each chapter and of their contents indicates that it does no such thing. In fact, this is yet another collection of review articles.

Because of this plethora of activity the editors of new haematological review series must exercise considerable ingenuity in finding topics which have not been covered many times in the previous year or two. In fact, there are one or two subjects which appear in this edition of *The Year in Hematology* which fall into this category, although not many. For example, the interesting chapters on the regulation of the egress of bone marrow cells, thymosin, and the effects of anti-cancer agents on haemopoietic progenitors deal with areas which have not been over-

exposed recently. On the other hand haemoglobin switching, cytogenetic studies in leukaemia, leukaemia antigens, and recent advances in the treatment of Hodgkin's disease and non-Hodgkin's lymphoma have all figured with monotonous regularity.

With a few exceptions the subjects covered in the 1978 *Year in Hematology* are dealt with thoroughly, referenced adequately and presented clearly. The book is well produced and illustrated. Perhaps it is unfair to judge this series after only two editions but the editors are certainly going to have

to use all their imagination to really justify the existence of yet another review series in a field in which the number of 'overviews' seems to be in danger of exceeding the number of original articles which are available to be reviewed. Indeed, the horrid neologism 'overview' seems to be in real danger of taking on a rather different meaning to that which its inventors had in mind!

D. J. Weatherall

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Green crop fractionation

Leaf Protein and Other Aspects of Fodder Fractionation. By N. W. Pirie. Pp. 183. (Cambridge University Press: Cambridge and London, 1978.) £8.

IMPROVED systems of crop production and utilisation attract world-wide attention. The technique of fractionation to produce high quality foods for humans and simultaneously feeds for farm livestock from fibrous green crops comes into this category. For approaching half a century N. W. Pirie has inspired, with his determination and enthusiasm, much research in both the developing and more developed regions of the world. Students, teachers, scientists and others with a devotion to improving food production, including those with particular interests in fractionation, would do well to read the book and to absorb the vast amount of investigation which has already taken place.

Leaf protein is potentially a most abundant source of protein with a good nutritive value, and temperate and tropical flora examined as sources of extractable protein are numerous. The separation and preparation of good fractionation products is well documented and these can be stored safely to prevent deterioration. A vast amount of qualitative information is already available from the scientific literature and the need now is for more quantitative data: crop and machines used must be thoroughly defined so that the end-products cease to be mysterious. The main challenge now is to assess when the exciting technical potential can be harnessed to provide worthwhile practical systems.

N. W. Pirie has always been interested in the less developed areas of the world and already there is evidence of sub-tropical villagers using leaf protein as food. The expansion of this type of work needs a missionary approach and substantial financial support to act as

the catalyst. Adoption of the technique has been slow. At the present time a greater interest seems to exist for exploiting the fractionation technique on a factory scale to produce leaf protein more as a source of pigment for poultry rations than as a source of protein. Some units already exist in Europe and North America and a modest expansion of this use of the technique is likely to occur around the world. Whether as a 'village' or a 'factory' operation the prospects for fractionation need discussion and debate. Further research is also required and a number of proposals are made in the chapters of this book.

Greater attention should be directed to improving the efficiency of green crop production and utilisation. Although it is technically possible by fractionation to produce leaf protein, we must not lose sight of the fact that some 80% of the harvested dry matter is best suited for ruminant consumption and certainly cannot be discarded. In many ways the use of fibrous green crops for human foods is exciting but there is a necessary balance between this and ruminant feeding.

Two centuries after the death of Hilaire Marin Rouelle, who published the first scientific papers on leaf protein, the world is certainly no less dependent on the politicians and the economic climates they create and foster. Progress has been made in green crop fractionation and the prospects are tantalising especially if scientists in the wake of N. W. Pirie can help the necessary biochemical engineering to take place and develop reliable systems robust enough to be profitable compared with alternatives irrespective of the scale and objectives of the operation. Investigation of the future prospects of green crop fractionation deserves support and the book is a timely publication.

J. Connell

J. Connell is Principal Scientific Officer at the National Institute for Research in Dairying, Shinfield, UK.

Gregory Rift Valley and early man

Geological Background to Fossil Man. Recent Research in the Gregory Rift Valley, East Africa. Edited by the late W. W. Bishop. (Scottish Academic Press: Edinburgh, 1978.) £25.

THE subtitle of this book is important, but it needs some explaining. The Gregory Rift Valley, for the benefit of non-geologists, is part of the great East African Rift—the system of valleys bounded by faults that extends southwards from the Red Sea for a distance of some 3,000 km. The section of this system known as the Gregory Rift, if one is to be precise, runs only for about 450 km from the bottom end of Lake Turkana in northern Kenya to the Lake Natron and Ngorongoro areas in northern Tanzania. But for the purposes of this book, the Gregory Rift has been interpreted more widely to include the northwards extension of the Rift—otherwise called the Ethiopian Rift—to the Afar in northern Ethiopia. The important early man sites at Hadar, and those in the Omo Valley and east of Lake Turkana thus come within the book's scope.

By geological accident, the Gregory Rift Valley—in both narrow and wide senses—is a field laboratory perhaps without equal for palaeontological and archaeological studies of early man as well as for investigating the structural, volcanic and geophysical aspects of rift systems. It was therefore a good idea of the late W. W. (Bill) Bishop's to organise in February 1975 a meeting at the Geological Society of London of many of the geologists and others who had worked or were still working in different parts of this great valley. This book—a collection of 35 papers—is the outcome. Not all the contributions are geological, but all in one way or another add to knowledge of the development of the Gregory Rift Valley and the environment of early man at different times in this part of East Africa.

A major part of the book is about the geology and palaeontology of the Baringo Basin. This was the area in which Bishop, and also Basil C. King—Director of the East African Geological Research Unit and Bishop's Professor for a period—devoted much of their own and their students' time between the mid-1960s and mid-1970s. It is a loss to science that with Bishop's tragically early death in 1977 and King's retirement from the chair of geology at Bedford College, London, systematic geological and palaeontological surveying in the Baringo area

has almost ceased, although an archaeological project at Chesowanja does continue.

As events have turned out, the book has become a memorial tribute to Bishop's geological work in East Africa. It is therefore a pity that the volume is not all that it might have been. Bishop unfortunately died before he had completed the introduction to the three main parts and also the summing-up, so the book lacks these sections—though this is not the publisher's fault. I was, however, sorry to see so many typographical errors and minor editorial inconsistencies for which the publisher should take responsibility. These are understandable in the circumstances, but are to be regretted nonetheless. I should also mention that 'recent' in the subtitle means no later than 1975–76.

Geological Background to Fossil Man remains, however, a useful record of research in the Gregory Rift Valley

and is especially valuable for the many geological maps and sections it contains. A special feature, and one that is rare these days, is the inclusion of a 1:10,000 coloured, foldout geological map of the area around Olorgesailie Prehistoric Site in Kenya. This map was prepared in 1946 by Robert M. Shackleton for the First Panafrican Congress of Prehistory in Nairobi but money was not available then for its publication. For this edition, grants towards the cost of publication have come from the Royal Society and the L. S. B. Leakey Foundation. The names of the stratigraphical units have been updated and the map was specially redrawn by Barbara Isaac. It accompanies a detailed account by Glynn Ll. Isaac of the geology of the Olorgesailie Formation. **Sarah Bunney**

Sarah Bunney was recently Editor at The International Louis Leakey Memorial Institute for African Prehistory in Nairobi, Kenya.

Ion-selective electrodes

Analysis with Ion-Selective Electrodes. By J. Vesely, D. Weiss and K. Stulik. Pp.243. (Wiley: New York, London and Sydney, 1978.) £16.

THE Ellis Horwood Series in Analytical Chemistry under the guidance of Dr R. A. Chalmers (Editor of *Talanta*) and Dr Mary Masson, of the University of Aberdeen, will be useful both to professional analysts, working in industry and the public services, and those few who teach analytical chemistry as a specialist subject in universities and polytechnics.

This volume is concerned with the applications of ion-selective electrodes as 'sensors' that instrument technologists will need to interface with microprocessors—for example, when monitoring sodium and potassium levels for hospital patients. The authors are from Prague, home of J. Heyrosky and polarography fame, which guarantees that their work rests on a good theoretical background. The translation is smooth and clear, so that English readers can easily follow their text.

The work is divided into four chapters; the last is long and devoted to analytical applications. The first two deal with introductory theory and instrumentation, in some seventy pages with more than 300 references. Exact treatments are given of the theory of membrane potentials and activities, classification of electrode types with details of their selectivities and response characteristics, and information about measuring instruments used in potentiometry. The third chapter gives

the experimental techniques used, including directions for the preparation and presentation of samples for analysis, descriptions of discrete and continuous measurements with explanations of calibration and standardisation techniques. This chapter finishes with an account of automated methods of analysis and the use of ion-selective electrodes in biochemistry, biology and medicine.

The final long chapter surveys the applications, as reported in the world's literature until about the end of 1977. It seems to be comprehensive; some 840 references are reviewed in 78 pages. These are dealt with rather in order of the reliability of present-day electrodes, starting with the applications for halogens and cyanide in greatest detail and continuing with sulphur compounds, nitrogen (including enzyme reactions), phosphorous and other non-metals more briefly. Cation-selective electrodes are treated in the same way, starting with alkali and alkaline earth metals, followed by silver, mercury and so on. The book continues with tabulations for the determination of various inorganic materials and organic compounds and concludes with an Appendix of useful data tables.

This then is a valuable text and one does not need to criticise such honest work on the part of the authors. However, it is hoped that at a later time they may be encouraged to include the glass pH electrode, purposely avoided in this volume, as it is still the 'best' ion-selective electrode.

J. A. W. Dalziel

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announcements

Meetings

4-18 March, **Comparative Biology of the Nervous System**, Caracas (Dr Guillermo Whitfembury, Director of CLAB, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas, Venezuela).

9-10 March, **New York Einstein Centennial Celebration** (Einstein Committee, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

12-16 March, **Labex International '79**, Birmingham (Industrial and Trade Fairs Ltd, Radcliffe House, Blenheim Court, Solihull, West Midlands, UK).

13-16 March, **Tunnelling '79**, London (Christopher Bradley Ltd, 78 Vineyard Hill Rd, Wimbledon Park, London SW1, UK).

15 March, **Charge Coupled Devices in Signal Processing**, London (Dr A. Holmes-Seidle, Fulmer Research Institute, Stoke Poges, Slough, UK).

27-29 March, **SEA-CON (79)**, Brighton (C. G. Hague, Kent Instruments Ltd, 27B Bell St, Reigate, Surrey, UK).

28-30 March, **Process Engineering—the Future**, Loughborough (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

1 April, **More Physics, Less Relevance?**, Cambridge (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3-5 April, **Annual Chemical Congress**, Bristol (Dr John F. Gibson, The Chemical Society, Burlington House, London W1, UK).

3-6 April, **3rd International Symposium on Distillation**, London (S. A. E. Buxton, The Institution of Chemical Engineers, 165-171 Railway Terrace, Rugby, UK).

9-11 April, **River Pollution Control**, Oxford (Conference Organiser, Water Research Centre, Medmenham Laboratory, Henley Rd, Medmenham, Bucks, UK).

9-11 April, **Enzyme Inhibitors as Drugs**, London (Mrs J. Kruger, Dept of Pharmacology, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1, UK).

9-11 April, **Pollution from Road Vehicles**, Warwick (NSCA, 136 North St, Brighton, Sussex, UK).

10-11 April, **Symposium on Straw Decay**, Hatfield (Dr E. Grossbard, Hatfield Polytechnic, Bayfordbury House, Lower Hatfield Rd, Hertford, UK).

16-21 April, **Freeze-etching in Electron Microscopy**, Woods Hole (Admissions Office, MBL, Woods Hole, Massachusetts 02543).

18-19 April, **Photochemistry, Lasers and Spectroscopy**, Newcastle upon Tyne (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V0, BN, UK).

2-4 May, **Drug Treatment in Chronic Cerebrovascular Disorders**, Milan (Dr G. de Gaetano, Istituto di Ricerche Farmacologiche "Maro Negri", 20157 Milano, Via Eritraea, 62 Italy).

8 May, **Efficient Use of Resources: Implications for the Agricultural Engineer**, Kenilworth (Mrs E. J. Holden, The Institution of Agricultural Engineers, West End Rd, Silsoe, Bedford, UK).

8 May, **International Symposium on Crop Protection**, Gent (Rijksuniversiteit Gent, Faculteit van de Landbouwwetenschappen, B-9000 Gent, Belgium).

9-11 May, **Water Research Centre Open Days**, Medmenham (WRC PO Box 16, Henley Rd, Medmenham, Marlow, Bucks, UK).

9-11 May, **From Molecules to Community: Multifaceted Mental Health Research**, Saskatoon (D. G. Irvine, Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan, Canada S7N 0W8).

27 May-1 June, **14th World Gas Conference**, Toronto (Conference Office, PO Box 1979, Commerce Court West, Toronto, Ontario, Canada M5L 1J4).

6-7 June, **Mechanisms of Viral Pathogenesis and Virulence**, Munich (P. A. Bachmann, WHO Collaborating Centre, Koniginstr. 49, 8000 Munich 22, FRG).

17-19 June, **21st National Annual Congress of the Argentine Neurosurgical Association**, Santa Fé (Dr C. Cortelezzi, Casilla de Correo 1936, Buenos Aires, Argentina).

18-20 June, **Processes and Water Quality Modelling**, New Orleans (Dr P. Hamilton, Science Applications Inc., 4900 Water's Edge Drive, Suite 255 Raleigh, NC 27606).

18-21 June, **Metabolic Effects of Alcohol**, Milan (Organising Secretariat, Centro Studi de' Alimentazione, Nutrition Foundation of Italy, Via S. Pietro Alliberto 17, 20121 Milan, Italy).

25-29 June, **Excess Electrons and Metal-Ammonia Solutions**, Aviemore (Dr B. C. Webster, Chemistry Dept, The University of Glasgow, UK).

Reports & publications UK & Ireland—December

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 284, No. 1002: The Biochemical Functions of Terpenoids in Plants. A Discussion organised by T. W. Goodwin, FRS. Pp. 437-603+3 plates. (London: The Royal Society, 1978.) UK £10.90; Overseas £11.40. [412]
Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fisheries Radiobiological Laboratory. Technical Report FRL 14: Radioactivity in Surface and Coastal Waters of the British Isles, 1976. Part 2: Areas Other Than the Irish Sea and Its Environs. By N. T. Mitchell. Pp. 20. (Lowestoft: Ministry of Agriculture, Fisheries and Food, Fisheries Radiobiological Laboratory, 1978.) [412]

Loughborough University of Technology: Department of Civil Engineering. Water and Waste Engineering for Developing Countries. 4th WEDC Conference: Engineering for Health in Hot Countries, 25th-27th September 1977. Edited by John Pickford. Pp. 192. (Loughborough, Leicestershire: Mrs Rowena Steele, Department of Civil Engineering, University of Technology, 1978.) Softback £8; Hardback £12. [412]
Third Report from the Select Committee on Science and Technology. Session 1977-78. The Durability and Efficiency of Filament and Discharge Lamps. Vol. 1: Report. Pp. xxxiii. 80p net. Vol. 3: Appendices to the Minutes of Evidence. Pp. vii+267-452. £3.10 net. (London: HMSO, 1978.) [512]
The Edinburgh School of Agriculture. Annual Report, 1977. Pp. 239. (Edinburgh: The Edinburgh School of Agriculture, West Mains Road, 1978.) [612]
Home Office. Report of the Committee on Data Protection. Chairman: Sir Norman Lindop. Pp. xxiv+460. (Cmd. 7341.) London: HMSO, 1978.) £6 net.

A Review of Auxiliary and Booster Fan Ventilation Practice in Mines. (A Report by the National Committee to Examine All Aspects of the Ventilation of Narrow Drivages.) Pp. 41. (London: HMSO, 1978.) £2.25 net. [712]
Department of the Environment; Scottish Office; Welsh Office. Annual Survey of Radioactive Discharges in Great Britain, 1977. Pp. 42. (London: Department of the Environment, Waste Management Division, Becket House, Lambeth Palace Road, 1978.) 70p. net. [712]
Department of Industry. Report of the National Committee on Computer Networks. Presented to the Secretary of State for Industry by J. Howlett, CBE. Pp. vi+121. (London: Department of Industry, Dean Bradley House, 52 Horseferry Road, SW1, 1978.) gratis. [712]
Annual Report of the British Council, 1977-78. Pp. 72. (London: The British Council, 10 Spring Gardens, SW1, 1978.) [712]

- Engineering Structures*, Vol. 1, No. 1, October 1978. Edited by Dr J. Munro and Professor P. L. Gould. Pp. 1-56. Published quarterly. Annual subscription: (4 issues) £35. Single copy £8.25. (Haywards Heath, Sussex: IPC (Sales and Distribution), Ltd., Oakfield House, Perrymount Road, 1978.) [712]
- Bulletin of the British Museum (Natural History). Entomology Series, Vol. 37, No. 6: The Phlebotomine Sandflies (Diptera: Psychodidae) of the Oriental Region. By D. J. Lewis. Pp. 217-343. (London: British Museum (Natural History), 1978.) £17.85. [812]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 285, No. 1003: The Separation of Visual Axes in Apposition Compound Eyes. By G. A. Horridge, FRS. Pp. 1-59 + plates 1-8. UK £6.10; Overseas £6.35. Vol. 285, No. 1004: The Constitutive Heterochromatin in Chromosomes of *Fritillaria* sp., as Revealed by Giemsa Banding. By L. F. La Cour, FRS. Pp. 61-71. UK £2.10; Overseas £2.20. (London: The Royal Society, 1978.) [812]
- Computer Board for Universities and Research Councils. (Report of the Computer Board for the period 1st April, 1976-31st March, 1977. Pp. 16. (Cmd. 7322.) (London: HMSO, 1978. 40p net. [812]
- The Analysis of Archaeological Insect Assemblages: A New Approach. By H. K. Kenward. (The Archaeology of York: Principles and Methods, Vol. 19, Fasc. 1.) Pp. 68 + 4 plates. (London: Council for British Archaeology, 112 Kennington Road, SE11, 1978.) £4.75. [1112]
- University of Bristol. Annual Report of Council to Court and Statement of Accounts, 1977/1978. Pp. 67. (Bristol: The University, 1978.) [1112]
- Home Office. Experimental on Living Animals: Statistics. Pp. 37. (Cmd. 7333.) (London: HMSO, 1978.) £1.25 net. [1112]
- National Radiological Board. NRPB-R77: Radiation Exposure of the UK Population. By F. E. Taylor and G. A. M. Webb. Pp. iv + 86. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1978. Obtainable from HMSO, London.) £2.50. [1112]
- Shift Work and Health: A Critical Review of the Literature. By J. M. Harrington. (Report commissioned by the Employment Medical Advisory Service of the Health and Safety Executive.) Pp. v + 28. (London: HMSO, 1978.) £1 net. [1212]
- Natural Environment Research Council. Report of the Council for the period 1 April 1977-31 March 1978. Pp. v + 126 + 19 photographs. (London: HMSO, 1978.) £3.50 net. [1312]
- Social Science Research Council. Annual Report, 1977/78. Pp. 48. (London: HMSO, 1978.) £1.15 net. [1312]
- Some Biological Transformations Involving Unsaturated Linkages: The Importance of Charge Separation and Charge Neutralization in Enzyme Catalysis. By M. Akhtar and C. Jones. (Tetrahedron Report No. 43.) Pp. 22. (Oxford and New York: Pergamon Press, 1978.) £4.95. [1312]
- Tetrahedron Reports. No. 51: Die Chemie des Nihydrins und Anderer Cyclicher 1, 2, 3-Tricarbonsverbindungen. Von Alexander Schonberg und Erich Singer. Pp. 19. £4.95. No. 52: β -Lactams—Retrospect and Prospect. By Arya K. Mukerjee and A. K. Singh. Pp. 40. £4.95. No. 53: Sterically Crowded Organic Molecules—Synthesis, Structure and Properties. By Thomas T. Tidwell. Pp. 17. £4.95. No. 54: Nucleophilic Displacement of Aromatic Nitro Groups. By James R. Beck. Pp. 15. £4.95. (Oxford and New York: Pergamon Press, 1978.) [1412]
- University of Surrey. Research Committee. Register of Research Projects, 1978. Pp. ii + 157. (Guildford: Bureau of Industrial Liaison, University of Surrey, 1978.) [1512]
- Electronics—The Lifeline. By Admiral of the Fleet, The Earl Mountbatten of Burma. (The First Mountbatten Lecture.) Pp. 20. (London: The National Electronics Council, Abell House, John Islip Street, SW1, 1978.) 60p. [1512]
- Report of the Science Research Council for the year 1977-1978. Pp. vi + 97. (London: HMSO, 1978.) £3.50 net. [1512]
- Natural Environment Research Council. Report of the Council for the period 1 April 1977-31 March 1978. Pp. v + 126. (London: HMSO, 1978.) £3.50 net. [1812]
- Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 78, No. 10: Integration, Variation and Differentiation in Division Spaces. By R. Henstock. Pp. 69-85. £1.56. No. 11: Correlation Functions for Spherical Harmonics Resulting from Rotational Brownian Motion of a Linear Molecule. By James McConnell. Pp. 87-97. £1.70. No. 12: An Estuarine Water-Quality Model in an Oscillating Constant-Volume Reference Frame. By J. P. O'Kane. Pp. 99-118. £2. No. 13: The Synatron—a New Integrated Element for Signal Processing. By W. D. Ryan and D. H. Campbell. Pp. 119-126. 90p. No. 14: A Topological Formulation of the Density Theorem for O-Primitive Near-Rings. By D. Ramakotiah and G. L. Rao. Pp. 127-135. £1.10. No. 15: Decompositions in Nuclear Spaces. By J. J. M. Chadwick. Pp. 137-141. 85p. Section B: Biological, Geological and Chemical Sciences. Vol. 78, No. 10: The Irish Fauna of Empididae (Diptera: Brachycera). Pp. 145-169. £1.80. No. 11: An Electrophoretic Study of the Haemoglobins of Some Hybrid Fishes. By T. F. Cross and F. J. O'Rourke. Pp. 171-178 + 9 plates. £1.14. No. 12: Littoral and Benthic Investigations on the West Coast of Ireland—IX (Section A: Faunistic and Ecological Studies.) The Biology of the Rock-Goby, *Gobius paganellus* L., at Carna. By J. Dunne. Pp. 179-191. £1. (Dublin: Royal Irish Academy, 1978.) [1812]
- Office of Population Censuses and Surveys. Mortality Statistics: Childhood and Maternity. Pp. x + 102. (Series DH3, No. 3.) £2.50 net. Mortality Statistics: Review of the Registrar General on Deaths in England

- and Wales, 1976. Pp. xii + 104. (Series DH1, No. 4.) £2.75 net. (London: HMSO, 1978.) [1812]
- The Royal Society: Scientific Research in Schools Committee. Report to Council, 1978. Pp. 13. (London: The Royal Society, 1978.) [1912]
- Microelectronics: The New Technology. Pp. 24. (London: Department of Industry, 123 Victoria Street, SW1, 1978.) [1912]
- Health and Safety Commission. Report 1977-78. Pp. v + 65. (London: HMSO, 1978.) £1.75 net. [1912]
- Highly Flammable Liquids in the Paint Industry. Pp. iv + 47. (London: HMSO, 1978.) £1 net. [2112]
- Radiochemicals 1979. Pp. 151. (Amersham: The Radiochemical Centre, 1978.) [2112]
- Edwards Freeze-Drying Handbook. By Terence W. G. Rowe and John W. Snowman. Pp. 139. (Crawley, West Sussex: Edwards High Vacuum, Manor Royal, 1978.) £8. [2112]

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- Institute of Hydrology. Research Report, 1976-78. Pp. v + 124. (Wallingford, Oxfordshire: Institute of Hydrology, Maclean Building, Crowmarsh Gifford, 1978.) [81]
- Medical Research Council. Annual Report, April 1977-March 1978. Pp. x + 96. (London: HMSO, 1978.) £3.75 net. [81]
- Mineral Resources Consultative Committee. Mineral Dossier No. 20: Bauxite, Alumina and Aluminium. Compiled by Dr. R. N. Crockett. Pp. 53. (London: HMSO, 1978.) £1.75 net. [81]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290, No. 1371: A Study of 5p Excitation in Atomic Barium. I. The 5p Absorption Spectra of Ba I, Cs I and Related Elements. By J. P. Connerade, M. W. D. Mansfield, G. H. Newsum, D. H. Tracy, M. A. Baig and K. Thimm. Pp. 327-352. plates 1 and 2. UK £2.10; Overseas £2.20. Vol. 290, No. 1372: Acoustic Destabilization of Vortices. By E. G. Broadbent and D. W. Moore. Pp. 353-371. UK £1.25; Overseas £1.35. (London: The Royal Society, 1979.) [81]
- International Computers Limited. Annual Report and Accounts, 1978. Pp. 32. (London: International Computers, Ltd., ICL House, Putney, SW15, 1979.) [91]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 284, No. 1001: Evolutionary Systematics of Bivalve Molluscs. A Discussion organized for the Royal Society and Malacological Society of London by Sir Maurice Yonge, F.R.S., and T. E. Thompson. Pp. 199-436 + 29 plates. (London: The Royal Society, 1978.) UK £16.50; Overseas £17. [121]

Other countries—December

- International Atomic Energy Agency Publications—Catalogue, 1978/79. Pp. 247. (Vienna: Atomic Energy Agency, Kartner Ring 11, 1978.) [412]
- Republic of the Sudan: Ministry of Agriculture, Agricultural Research Division. Annual Report of the Gezira Research Station and Substations, 1966-1967. Pp. 353. Annual Report of the Hudeiba Research Station, 1972-1973. Pp. 63. (Wad Medani: Gezira Research Station, Agricultural Research Corporation, 1978.) [412]
- Fisheries and Environment Canada. Fisheries and Marine Service Technical Report No. 801: A Review of Water Reconditioning Re-use Technology for Fish Culture, with a Selected Bibliography. By T. Pettigrew, E. B. Henderson, R. L. Saunders and J. B. Sochasky. Pp. iv + 19. (St. Andrews, NB: Biological Station, Fisheries and Oceans Canada, 1978.) [412]
- Annals of the South African Museum. Vol. 76, Part 2: The Evolution of Guinea-Fowl (Galliformes, Phasianidae, Numidinae) Taxonomy, Phylogeny, Speciation and Biogeography. By T. M. Crowe. Pp. 43-136. Vol. 76, Part 5: The Skeleton of the Mammal-Like Reptile *Cistecephalus* With Evidence for a Fossorial Mode of Life. By Michael A. Cluver. Pp. 213-246. Vol. 76, Part 7: Late Tertiary Hyacinthidae from Langebaanweg, South Africa, and Their Relevance to the Phylogeny of the Family. By Q. B. Hendey. Pp. 265-297. Vol. 76, Part 8: A Fragmentary Specimen of *Saurichthys* SP. from the Upper Beaufort Series of South Africa. By John Griffith. Pp. 299-307. Vol. 76, Part 9: Two New Species of *Gastrosaccus* (Crustacea, Mysidacea) from Sandy Beaches in Transkei. By T. Wooldridge. Pp. 309-327. Vol. 76, Part 10: Late Tertiary Mustelidae (Mammalia, Carnivora) from Langebaanweg, South Africa. By Q. B. Hendey. Pp. 329-357. (Cape Town: South African Museum, 1978.) [412]
- SINET: An Ethiopian Journal of Science. Vol. 1, No. 1, June 1978. Edited by Ermas Dagne. Pp. 1-68. (Addis Ababa: Faculty of Science, Addis Ababa University, PO Box 1176, 1978.) [512]
- Wattle Research Institute. Report for 1977-1978 (Thirty-first Year). (Wattle Bark Industry of South Africa: South African Timber Grower's Association; University of Natal; State Department of Forestry; Forestry Council; Forest Owners' Association.) Pp. 142. (Pietermaritzburg: Wattle Research Institute, University of Natal, 1978.) [612]
- Annals of the South African Museum. Vol. 76, Part 6: The Development of *Xenopus gilli* Rose and Hewitt (Anura, Pipidae). By R. E. Rau. Pp. 247-263. Vol. 77, Part 1: The South African Museum's *Meiring Naude* Cruises. Part 8: Isopoda Anthuridea. By Brian Kensley. Pp. 1-25. (Cape Town: South African Museum, 1978.) [612]
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